CIBA FOUNDATION COLLOQUIA ON ENDOCRINOLOGY

Vol. 10 Regulation and Mode of Action of Thyroid Hormones

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CIBA FOUNDATION COLLOQUIA ON ENDOCRINOLOGY

VOLUME 10

Regulation and Mode of Action of Thyroid Hormones

Editors for the Ciba Foundation

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PREFACE

As the Ciba Foundation had included fifteen conferences on endocrinological subjects in its first forty small, international and multi-disciplinary meetings, with only slight reference to the thyroid, it was clearly high time to devote a colloquium entirely to the subject. In a three-day meeting of our usual fairly relaxed conversational type, it was impossible even then to consider all aspects of thyroid physiology, and it was decided to concentrate our attention at one end on the regulation of the thyroid gland, and at the other on the character and mode of action of its hormones.

This colloquium would not have taken place without the interest, support and advice of Sir Charles Harington and Dr. Rosalind Pitt-Rivers, and in the event all members of the conference enjoyed and admired the chairmanship of Dr. Pitt-Rivers, the first lady to occupy this position on one of these occasions.

The facilities of the Ciba Foundation are such as to compel us to limit membership to about thirty, always a distressingly hard task of selection. Our experience fully confirms that in our circumstances a group of up to thirty in size can converse in a coherent, co-operative manner, whereas above this number people tend to remain isolated in their separate nationalities, disciplines and opinions. In a large company, members are also much more guarded in their expressions, for fear of offending unknown people, where it is not possible easily to be aware of the identities of all people present.

For those who could not be invited to join us at this meeting, the papers and the discussions they aroused, are reproduced extensively in this volume. We hope that the reader will obtain from it not only information and ideas, but also a sense of participation in a friendly occasion.

Although this will be the thirty-fourth book containing the

papers and discussions of one of the Ciba Foundation's conferences, it may be helpful to add a few explanatory words about the Foundation and its other activities. It is an international centre, established as an educational and scientific charity under the laws of England. It owes its inception and support to its Founder, CIBA Ltd. of Switzerland, but is administered independently and exclusively by its distinguished British Trustees.

The Foundation provides accommodation for scientific workers who visit London from abroad, organizes and holds international conferences, conducts (in conjunction with the Institut National d'Hygiène) a postgraduate medical exchange scheme between England and France, arranges informal meetings for discussion, awards two annual lectureships, has initiated a scheme to encourage basic research relevant to the problems of ageing, assists international congresses and scientific societies, is building up a library service in special fields, and generally endeavours to give aid in all matters that may promote international co-operation in scientific research.

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List of those participating in or attending the Colloquium on "Regulation and Mode of Action of Thyroid Hormones" 20th–22nd June, 1956

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CHAIRMAN'S OPENING REMARKS

ROSALIND PITT-RIVERS

MY first duty is a very agreeable one to thank the Ciba Foundation on behalf of us all for arranging this symposium on the regulation and mode of action of thyroid hormones. This is the first of the Ciba Foundation meetings which has been devoted exclusively to the thyroid gland, and it comes at a time most opportune for us; we can now take stock of some of the discoveries in this field which have been made in the past ten or fifteen years.

During this time the use of the radioactive isotope of iodine ^{131}I has contributed largely to our knowledge. It has allowed us to study dynamic aspects of iodine metabolism in man and animals in health and disease and to investigate alterations in thyroid function as influenced by other endocrine organs. The part played by the hypothalamus in the control of the thyroid by the anterior pituitary has received special attention, and the relationship of the adrenals and of the pancreas to certain aspects of thyroid function is now beginning to be investigated.

In the biochemical field, chromatography of ^{131}I-labelled thyroid products has enabled us to detect and separate iodinated compounds which are present in biological material in amounts too small to be investigated by the older analytical methods These studies have led to the discovery of 3 : 5 : 3′-triiodothyronine, which joins thyroxine as one of the thyroid hormones.

Recently two other iodinated thyronines have been demonstrated in the thyroid and in other tissues, further, tetraiodothyroacetic acid (TETRAC) and triiodothyroacetic acid (TRIAC), which have been postulated as likely metabolites of thyroxine and triiodothyronine, have been detected in

tissues and tissue homogenates after administration of the parent amino acids *in vivo* and *in vitro*. Whether these new compounds also have a place among the thyroid hormones is not yet established, but they merit further investigation.

In spite of the large amount of work which has been done on the mode of action of thyroid hormones, we are still in the dark as to its exact nature. Much of this effort has been directed to the investigation of effects at a sub-cellular level and it is now thought that one of the thyroid's actions is to control the liberation of energy released during biological oxidations. It does indeed seem reasonable that hormones which contribute so much to the control of energy in the whole animal should have as their target some part of the control mechanism of coupled high energy phosphate reactions. It is however unlikely that this is the whole story, and future work may show that the overall thyroid hormone effect is only manifested in a physiological system which includes other endocrine glands, since these exert so great an influence on thyroid function *in vivo*.

One result of the spurt in thyroid research has been some divergence of opinion about a number of problems. The Ciba Foundation now offers us an opportunity to air these differences and help to resolve them by an exchange of information and by discussion.

HYPOTHALAMUS-PITUITARY-THYROID RELATIONSHIPS*

G. W. Harris and J. W. Woods

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Previous work (Brown-Grant, von Euler, Harris and Reichlin, 1954; Brown-Grant, Harris and Reichlin, 1954*a*, *b*), which was briefly reported by Harris (1955*a*) at a Ciba Foundation Colloquium, demonstrated that the ^{131}I output method forms a simple and direct technique for observing changes in thyroid activity in the rabbit. The necessary procedures may be carried out without anaesthesia and afford a period of 10–18 days, in any one experiment, in which changes in thyroid activity may be detected. In the work cited above, it was found that with the animals under standardized conditions the rate of release of thyroidal radioactivity follows an exponential curve and that a decreased or increased activity of the gland is associated with a decreased or increased slope of the curve respectively. Stressful or noxious stimuli were uniformly found to produce inhibition of the thyroid gland, and evidence was adduced that this effect was mediated by the hypothalamus and anterior pituitary gland. Since such stimuli uniformly lead to increased activity of the adrenal cortex it was pointed out that under many, if not all, conditions of environmental change a reciprocal relationship seemed to be maintained between the thyroid and adrenal cortex. A study of the effect of administration of adrenocorticotrophic hormone (ACTH) or cortisone on thyroid activity in the adrenalectomized rabbit showed that inhibition of the thyroid gland occurred when the blood concentration of adrenal corticoids was increased.

* The original work reported in this paper was carried out during the tenure by one of us (J. W. W.) of a Fellowship of the American Cancer Society, and aided by a grant from the Research Fund of the Maudsley Hospital.

The aim of the present experiments was to investigate any changes in thyroid activity produced by prolonged electrical stimulation of the hypothalamus in the unanaesthetized rabbit. Green and Harris (unpublished, cited by Harris, 1955*b*) stimulated various regions of the hypothalamus and pituitary gland of unanaesthetized rabbits and studied their oxygen uptake No consistent changes were observed but the limitations of this method for assessing thyroid activity prevented any definite conclusions from being drawn. Colfer (1949) *found histological signs of increased thyroid activity* in rats and rabbits after prolonged electrical stimulation of the hypothalamus. An increased discharge of thyrotrophic hormone (TSH) was reported by Ellis and Wiersma (1945) following repeated electronarcosis in dogs and guinea pigs. Del Conte, Ravello and Stux (1955) confirmed these findings in the guinea pig and reported a rise in blood concentration of TSH within 30 *minutes* of electroshock. In view of these data and the well-established fact that electrical stimulation of the region of the median eminence of the hypothalamus evokes anterior pituitary secretion of ACTH (rabbits, de Groot and Harris, 1950; dogs, Hume and Wittenstein, 1950; cats and monkeys, Porter, 1953, 1954), and of gonadotrophic hormone (Harris, 1937, 1948; Markee, Sawyer and Hollinshead, 1946) it seemed reasonable to suppose that similar stimulation might evoke discharge of TSH.

The method used for detecting changes in thyroid activity of the rabbit, in the present study, is the ^{131}I output method as described previously Electrical stimulation is performed by a modification of the remote control method of de Groot and Harris (1950). This technique makes it possible to stimulate the hypothalamus of the unanaesthetized, unrestrained rabbit. A small secondary coil is implanted subcutaneously over the lumbar spine and connected by flexible leads to glass-insulated platinum electrodes inserted into the hypothalamus or pituitary region. During an experiment the animals are housed in cages surrounded by a large primary coil, and stimulation applied at appropriate times by passing condenser

discharges through the field coil at 50 cyc./sec. The pulses induced in the secondary are alternating with a duration 0·5–0·7 m.sec. The primary circuit is provided with an interrupter which makes it possible to stimulate with on : off periods of any desired ratio—¼ min. on : ¼ min. off in the present experiments. The intensity of stimulation is controlled by the position of the rabbit's cage relative to the primary coil and by the capacitance of, and voltage applied to, the condenser bank discharging through the primary coil.

Results

(a) *Before complete adrenalectomy*

Electrical stimulation has been applied to the hypothalamus or pituitary gland of 27 animals in 41 experiments. Fifteen of

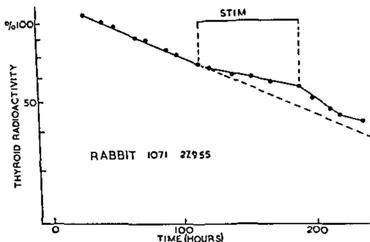


FIG 1. To show the effect of electrical stimulation of the tuber cinereum of the normal rabbit on the rate of release of ^{131}I from the thyroid gland

these rabbits were normal, 5 had been subjected to ovariectomy and right adrenalectomy, and 7 to right adrenalectomy. Stimulation for periods of several days (usually 48 hr) resulted in transient inhibition of thyroid activity in 22 experiments (Figs 1 and 4), no change in 16, a questionable

response in 1, and in only 2 experiments (on one normal rabbit) was a definite increase in thyroid function seen. In no case was a detrimental effect on the rabbits observed.

(b) *After complete adrenalectomy*

In view of the data indicating first, that increased ACTH secretion may follow electrical stimulation of the hypothalamus, and secondly, that an increased blood concentration

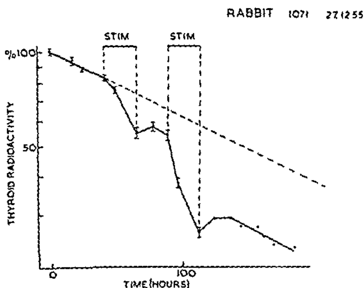


FIG 2. The effect of hypothalamic stimulation on ^{131}I release from the thyroid after adrenalectomy

of adrenal corticoids inhibits the secretion of TSH, it was decided to repeat the experiments in completely adrenalectomized rabbits maintained on constant daily doses of cortisone. Eighteen of the 27 rabbits reported above have been studied after complete adrenalectomy, and a striking change in the thyroid response has been observed in many cases. Forty-eight experiments on these animals showed that 12 rabbits now responded to the electrical stimulation with a marked

increase in thyroid activity (Figs. 2 and 3) (previous to adrenalectomy, they had shown thyroid inhibition in 10 experiments, and no change in thyroid activity in 6 experiments). The increase in thyroid function was assessed not only by measuring the rate of loss of thyroidal radioactivity, but in 5 experiments on 4 rabbits by simultaneous measurements of the total plasma radioactivity or the plasma $PB^{131}I$.

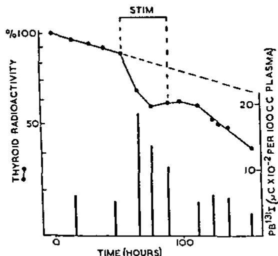


FIG 3 The effect of hypothalamic stimulation on ^{131}I release from the thyroid, and on the $PB^{131}I$ concentration in the plasma

The results indicated that the blood concentration of thyroid hormone was increased up to four-fold (Fig. 3). It is significant that hypothalamic stimulation maintained, in some cases, an increased level of thyroid activity even in the presence of a raised concentration of thyroid hormone in the blood.

The precise localization of the region stimulated has not yet been determined by histological study in all the animals, but

response in 1, and in only 2 experiments (on one normal rabbit) was a definite increase in thyroid function seen. In no case was a detrimental effect on the rabbits observed.

(b) *After complete adrenalectomy*

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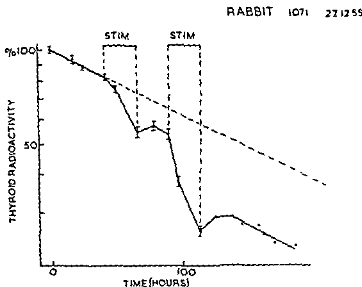


Fig. 2 The effect of hypothalamic stimulation on ^{131}I release from the thyroid after adrenalectomy.

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FIG. 5 Microphotographs of a horizontal section through the hypothalamus of rabbit 1074 Bi-polar electrodes have been implanted, the site of one electrode (x) may be seen as a circular hole in the anterior part of the median eminence The site of the other electrode (also x) was traced through serial sections and found to be in the

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the available studies (see Fig. 5) and the radiographic evidence suggest that it is stimulation of the anterior part of the median eminence of the tuber cinereum which results in increased thyroid activity.

Five of the rabbits that showed a marked acceleration of thyroid activity during stimulation eventually died, suddenly and unexpectedly, whilst being stimulated.

(c) *Cortisone-treated animals*

Seven rabbits with the left adrenal gland intact were given high daily doses of cortisone (5–10 mg.) in an attempt to at

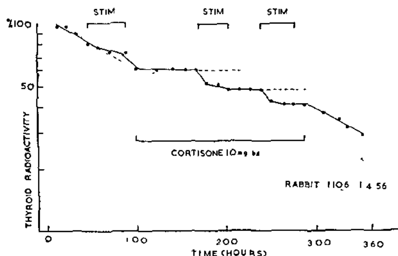


Fig 4 To show the effects of hypothalamic stimulation before and during cortisone administration on a rabbit with the left adrenal gland intact. Note (1) the reduced rate of release during the control periods whilst under cortisone administration, and (2) the reversal by cortisone of the effect produced by hypothalamic stimulation.

least partially blockade ACTH secretion, and subjected to electrical stimulation. Four of these animals in 6 experiments responded with an increased rate of release of thyroidal radioactivity (Fig. 4).

Discussion

The reversal of the thyroid response to electrical stimulation of the tuber cinereum after adrenalectomy may be explained in several ways. The most likely hypothesis seems to be that some substance is liberated from the adrenal glands during hypothalamic stimulation, which in turn inhibits either TSH secretion or the thyroid gland itself. This substance might originate in the adrenal medulla or cortex, since it is known that either adrenaline (Haigh, Reiss and Reiss, 1954; Brown-Grant, Harris and Reichlin, 1954*a*; Brown-Grant and Gibson, 1956) or adrenal cortical steroids (Myant, 1953; Brown-Grant, Harris and Reichlin, 1954*b*) may inhibit thyroid activity in the rabbit. It is unlikely that adrenal medullary secretion is involved since (a) localized stimulation of the tuber cinereum probably does not evoke secretion of adrenaline (Magoun, Ranson and Hetherington, 1937), (b) the dose of adrenaline necessary to produce prolonged inhibition of the thyroid gland of the rabbit is high (Brown-Grant, Harris and Reichlin, 1954*a*), and (c) rabbits with an adrenal gland intact may respond to stimulation with increased thyroid function if under high cortisone administration. It is probable that adrenal cortical secretion is the factor involved, since (a) localized stimulation of the tuber cinereum results in increased secretion of ACTH (de Groot and Harris, 1950; Hume, 1952), (b) the dose of adrenal steroids necessary to produce prolonged inhibition of the thyroid gland in the rabbit seems within the physiological range (Brown-Grant, Harris and Reichlin, 1954*b*, Brown-Grant, 1956), and (c) rabbits with an intact adrenal in which ACTH release is presumably blocked with large doses of cortisone show a thyroid response. It is probable that adrenal steroids affect thyroid activity by suppressing the secretion of TSH from the anterior pituitary, since cortisone was found not to influence the response of the thyroid gland of the hypophysectomized rabbit to injection of exogenous TSH (Brown-Grant, Harris and Reichlin, 1954*b*).

Worcester, Christo, Campbell and Zygmuntowicz, 1951) although the formaldehydogenic steroids are decreased (Daughaday, Jaffe and Williams, 1948). Daughaday and co-workers (1948) also report that a severe exacerbation of the thyrotoxic state was accompanied by a fall in the excretion of formaldehydogenic steroids and such an observation provides a rational basis for the treatment of thyroid crisis with administration of cortisone. In a more recent study Levin and Daughaday (1955) found the excretion of urinary 17-keto- and 17-hydroxysteroids to be within the normal range in hyperthyroidism. The surprising factor, on *a priori* consideration, is that urinary excretion of adrenal steroids is not greatly increased in Graves's disease, since the administration of exogenous thyroxine results in adrenal hypertrophy (Wallach and Reineke, 1949; and others), an increased excretion of 17-hydroxysteroids in the guinea pig (Levin and Daughaday, 1955) and of 17-ketosteroids in man (Corvilain, 1953). The physical and mental states of thyrotoxic patients would also seem to afford a strong stimulus to adrenal cortical hyperfunction.

3 In an investigation on thyroid-adrenocortical relationships in man, Hill, Reiss, Forsham and Thorn (1950) found that administration of ACTH or cortisone depressed the uptake of ^{131}I by the thyroid and lowered the concentration of blood protein-bound iodine, although they noted that exogenous TSH was still active in the presence of a high concentration of adrenal steroids in the blood. They also observed that ACTH and cortisone therapy might be of benefit to patients with Graves's disease and that the best results were obtained in early cases and in those with a good adrenal response to ACTH.

4. Marine (1930) reported that post-mortem examination of thyrotoxic patients revealed the presence of a small adrenal cortex, and such signs of adrenal hypofunction as lymphoid hyperplasia and a large thymus gland. LeCompte (1949)

The present findings are of interest in a consideration of the aetiology of Graves's disease, for two reasons.

First, the fact that localized stimulation of the rabbit's hypothalamus can result in increased thyroid activity is compatible with the thesis expressed in so many clinical accounts that emotional stress is an important aetiological factor in the onset of Graves's disease. The occurrence of hyperthyroidism following a severe fright was mentioned by Parry (1825) when he first described the disease. The publications of Crawford (1897), Maranon (1921), Deutsch (1923), Lewis (1925), Bram (1927), Moschcowitz (1930), Goodall and Rogers (1933) may be quoted from amongst the large number dealing with this subject. Further reference to the literature on the relationship of psychic trauma to Graves's disease may be found in the more recent papers of Lidz and Whitehorn (1950) and Mandelbrote and Wittkower (1955).

Secondly, the fact that stimulation of the hypothalamus is effective in the adrenalectomized rabbit rather than the normal is compatible with much data indicating a state of absolute or relative deficiency of the adrenal cortex in patients with Graves's disease. Emphasis was first placed on this point by Marine and Baumann (1921) and Marine (1930). The data at the present time may be summarized as follows:—

1. The occurrence of thyrotoxicosis in cases already suffering from Addison's disease has been reported (Rossle, 1914), and the frequency of onset of Graves's disease under these circumstances has been given as ten times that in previously normal subjects (Frederickson, 1951). Oppenheimer (1937) described the onset of hyperthyroidism following X-ray damage to the adrenal cortex in man.

2. The excretion of 17-ketosteroids seems reduced in patients with Graves's disease (Fraser, Forbes, Albright, Sulkowitch and Reifenstein, 1941; Shadaksharappa, Calloway, Kyle and Keeton, 1951; Corvilain, 1953). The excretion of reducing steroids may be normal or slightly increased (Shadaksharappa, Calloway, Kyle and Keeton, 1951; Talbot, Wood,

DEUTSCH, F. (1923). *Med. Klinik*, 19, 678.
ELLIS, C. H., and WIERSMA, C. A. G. (1945) *Proc. Soc. exp. Biol*, N.Y., 58, 160.
FRASER, R. W, FORBES, A. P, ALBRIGHT, F., SULKOWITCH, H., and REIFENSTEIN, E. C., Jr. (1941). *J. clin. Endocrin*, 1, 234.
FREDERICKSON, D. S. (1951) *J. clin. Endocrin.*, 11, 760.
GOODALL, J. S., and ROGERS, L. (1933). *Med. J. Rec*, 138, 411.
GROOT, J DE, and HARRIS, G. W. (1950). *J. Physiol.*, 111, 335.
HAIGH, C. P., REISS, M, and REISS, J. M. (1954). *J. Endocrin.*, 10, 273.
HARRIS, G. [illegible]
HARRIS, G. [illegible]
HARRIS, G. [illegible] 8, 531.
HARRIS, G. W. (1955*b*) Neural Control of the Pituitary Gland. London: Edward Arnold.
HILL, S. R., REISS, R. S, FORSHAM, P. H., and THORN, G. W. (1950). *J. clin. Endocrin.*, 10, 1375.
HUME, D. M. (1952) Ciba Foundation Colloquium on Endocrinology, 4, 87. London: Churchill
HUME, D M., and WITTENSTEIN, C J (1950). Proc. 1st Clin. ACTH Conf., p. 134. ed. J. R Mote. Philadelphia: Blakiston Co
KRAUS, E. J (1923). *Virchows Arch*, 247, 421.
LECOMPTE, P M (1949). *J. clin. Endocrin*, 9, 158.
LEVIN, M. E., and DAUGHADAY, W. M. (1955) *J clin Endocrin*, 15, 1499
LEWIS, N D. C (1925). *Med. J Rec.*, 122, 121.
LIDZ, T, and WHITEHORN, J C (1950). *Res. Publ. Ass. nerv. ment. Dis.*, 29, 445.
MAGOUN, H. W., RANSON, S. W., and HETHERINGTON, A. (1937). *Amer. J. Physiol.*, 119, 615.
MANDELBROTE, B. M, and WITTKOWER, E. D. (1955). *Psychosom. Med.*, 17, 109
MARAÑON, G. (1921), *Ann Méd.*, 9, 81
MARINE, D (1930). *Amer. J. Sci.*, 180, 767.
MARINE, D., and BAUMANN, E. J (1921). *Amer J. Physiol*, 57, 135.
MARKEE, J. E., SAWYER, C. H, and HOLLINSHEAD, W. H (1946). *Endocrinology*, 38, 345.
MOSCHCOWITZ, E. (1930). *Arch intern. Med.*, 46, 610.
MYANT, N. B. (1953). *J. Physiol*, 120, 288.
OPPENHEIMER, B S (1937) *J. Mt. Sinai Hosp.*, 4, 214
PARRY, C. H (1825) Elements of Pathology and Therapeutics, 2, 111.
PORTER, R. W. (1953). *Amer. J Physiol.*, 172, 515.
PORTER, R. W. [illegible]
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measured the width of the adrenal cortex and found a significant narrowing in cases of Graves's disease. Kraus (1923) describe... similarity of the human pituitary in cases of Addison ... ...osis. Perhaps more significant in ... (1935) that the thyroid gland in cases ... be histologically similar to that of Graves's disease. ... 944) summarizes the position by stating: "The more completely the matter is studied the more clearly does an underlying relationship become evident between three such apparently different conditions as exophthalmic goiter, status lymphaticus and Addison's disease."

It is likely that the normal human, as well as laboratory animals, responds to emotional or physical stress with an increased activity of the adrenal cortex. It is possible that if, for some reason, the adrenal cortical response fails to occur, and the inhibitory effect of a raised blood concentration of adrenal corticoids on TSH secretion is lacking, thyroid hyperactivity may ensue.

REFERENCES

Boyd, W. (1944) The Pathology of Internal Diseases. 4th Ed. London: *Henry Kimpton*

... W., and Reichlin, S.

... *J. Physiol.*, 131, 85

... ichlin, S (1954*a*). *J.*

... ichlin, S (1954*b*). *J.*

... p 376.

Crawford, ... 3, 45.

Crooke, A. C., and Russell, D. S. (1935). *J. Path. Bact.*, 40, 255.

Daughaday, W. M., Jaffe, H., and Williams, R. H. (1948). *J. clin. Endocrin*, 8, 244.

Del Conte, E., Ravello, J. J., and Stux, M. (1955). *Acta endocr., Copenhagen*, 18, 8.

after administering compounds B and F to normal rabbits. This action

Greer: The action of cortisone on the thyroid is very puzzling; there is evidence on both sides

Harris: The evidence regarding normal rats is clearer though. Many workers have found cortisone depresses the thyroidal uptake of ^{131}I in this form, and of more significance, Brown-Grant (1955. *Endocrinology*, 56, 607; 1956 *J. Physiol.*, 131, 58) has found that the same adrenal steroid, and also compounds B and F, decrease the rate of loss of thyroidal radioactivity.

B.-Grant: Yes, large doses of adrenal steroids result in a decrease in the rate of release of thyroidal radioiodine. The point I should like to make, though, is that the whole problem of the action of cortisone is a very complex one, as Dr. Greer said There are reports, for instance, that long-continued cortisone treatment in normal rats may produce thyroid hypertrophy (Winter, C. A., Silber, R H., and Stoerk, H. C. (1950) *Endocrinology*, 47, 60), although in acute experiments there is little doubt that it produces a decrease in thyroid activity.

I imagine most of the goitrogen experiments, too, are done on animals on an iodine-deficient diet So perhaps the initial effect of cortisone in

try.

B.-Grant: I do not think it is established that the effect of ACTH administration is mediated by any of the known corticoids produced from the adrenal. I say that particularly because, although the rabbit does produce F under conditions of continuous administration of ACTH, it mainly produces B which is not very effective when tested in the rabbit.

DISCUSSION

iodine in animals and man given large doses of cortisone, yet there is n
inhibition of the goitre produced by the administration of antithyroi

to be the case, then your point is why was no inhibitory action of the adrenocortical hormones demonstrated in the experiments involving the administration of goitrogens. I should like to ask first, though, how fa it would have been possible to measure or observe any inhibition if such had been present.

Res. Publ. Ass. nerv. ment. Dis., **29**, 451); Mandelbrote, B. M., and Wittkower, E. D. (1955. *Psychosom. Med.*, **17**, 109)) describing the frequency with which an emotionally stressful experience is found … It is generally said that about 80

innumerable reports I feel that the possibility that a stress serves in some way to trigger the onset of Graves's disease deserves serious consideration.

… incidence

… I. *J. clin. Endocrin.*, **11**, …

Albert: If thyrotoxicosis is actually due to pituitary stimulation in

…

there.

Purves: At Dunedin we have investigated the rôle of TSH in Graves's disease; TSH can be found in thyrotoxic cases—it is probably elevated above normal—but the fact that it is present, I would say is an

It may be that these effects are produced by some steroid or steroids that are present in very small amounts—perhaps a specific "thyrocortin" more active in this way than the known steroids This would perhaps fit in with Prof Harris's observations on the effect of adrenalectomy—the effect is not due to getting rid of the B or the F but some other factor that the adrenal produces.

Maclagan: Yes, such as the androgens.

B.-Grant I don't favour androgens in the rabbit, because large doses of testosterone, at any rate, do not have any significant effect on the thyroid activity of the rabbit, male or female, as far as the release curve is concerned (Brown-Grant, K. (1955). *J. Physiol*, 123, 390).

Maclagan Testosterone, of course, is not an adrenal androgen. It might, perhaps, be advisable to try an androgen *known to come from* the adrenal.

B.-Grant Yes, certainly that would be worth trying

Halmi: I was very pleased to hear that one gets thyroid enlargement if one gives cortisone long enough Dr. Barker and I have found that with cortisone treatment you can actually get an enlargement of cells in the thyroid. This certainly does not indicate an inhibition of release of TSH.

Taurog Is that in a normal or in a hypophysectomized animal?

Halmi That was in the "normal" animal I do not know how normal the animal actually was because the diet may have contained some goitrogen.

Harris For how long did you administer the adrenal steroids?

Halmi We had two groups of animals; in one group we gave them 5 mg. cortisone daily for 20 days, and the other group we gave 2 mg. cortisone for almost 3 months We got the same hypertrophic change in the thyroid cells in both groups

B.-Grant Have you tried giving iodide to these animals along with the cortisone?

Halmi No, we have not This is certainly a good suggestion; there may be an excessive loss of iodine in cortisone-treated rats

Barker I do not know if anyone has looked at how much iodide can move through the kidney of the rat; there certainly is grossly increased urinary excretion after administration of iodide to animals receiving cortisone treatment.

Albert. I should like to change the discussion in the direction of Prof. Harris's second point, namely the relationship of his findings to the aetiology of Graves's disease There are several points that I stumbled over. I do not think the epidemiology of Graves's disease is such that there are really good data concerning emotional stress to support this concept.

Harris: I have little personal experience in this field, and was merely quoting the general consensus of opinion as given in the literature. There are very many papers (amongst the recent ones are those of Lidz, T., and Whitehorn, J. C. (1950 *Res. Publ. Ass. nerv. ment. Dis.*, 29, 445), Lidz, T. (1953. *J. Mt. Sinai Hosp.*, 20, 27; 1955 *Psychosom. Med.*, 17, 420); Ham, G. C., Alexander, F., and Carmichael, H T. (1950.

adrenal secretes much compound E.

Purves: Have you done the experiment in the adrenalectomized rabbit of administering cortisone to see if that inhibits a response to stimulation which was obtained without cortisone?

histological evidence of depressed thyroid activity in the rat; the rabbit behaves similarly. But, in my experience, even such severe forms of "stress" as death from diphtheria, or from gas gangrene secondary to a

Prof. Harris said that the thyroid in myxoedema resembles the thyroid in thyrotoxicosis It may be that this occurs when there is a relatively greater depression of adrenocortical than thyroid function leaving the thyroid incompletely opposed

cortisone.

indication that the pituitary function is abnormal, for if the pituitary function were normal, the elevated thyroxine levels would have inhibited TSH. The fact that TSH does circulate in thyrotoxics is an indication that the thyroid activity in these cases is due to TSH.

Querido: I congratulate Prof Harris on the way he has been able to change his experimental preparation to obtain a positive response on stimulus. This only shows that he probably has a preparation where you can achieve thyroid function change by increased TSH production. But this is a long way from saying that the gland is a thyrotoxic gland. I am only thinking, for instance, of the point of the possibility of inhibition of the glands with the Werner test, which is pointing to a process of uncoupling. This does not necessarily mean that if you did the experiment long enough you would not achieve such a state.

Purves: We find that the rebound of ^{131}I levels after TSH stimulation is probably due in fact to a re-accumulation of iodide which is released from the thyroid by TSH—TSH releases not only hormone but also iodide, and when the blood level is increased by TSH stimulation of the thyroid, the blood when fractionated shows an increase both in iodide ^{131}I and hormonal ^{131}I, and the iodide is immediately available for re-accumulation

Harris: You mean this is iodide derived from degraded hormone?

Purves: No, direct from thyroid presumably.

Harris: What is the evidence that it is from the thyroid rather than from degraded hormone?

Purves: The iodide appears simultaneously with the thyroxine release.

Albert: Concerning the activity of the adrenal, I think that one can take data to indicate that patients with Graves's disease probably have a greater adrenal activity since in order to maintain a normal blood level of corticosteroids in the face of an accelerated disappearance rate (which has been experimentally verified), the adrenal would have to work faster.

Purves: I wonder if it is possible that the adrenal medulla may be involved in the inhibition in the animal with intact adrenals after the electrode implantation It does seem from your results, Prof. Harris, that where cortisone was given to an animal which did not give a response before but gave this thyroid stimulation after cortisone, cortisone from the adrenal could not be implicated as an inhibitory factor in the entire animal. On the other hand, this does not fit any better with the idea that the adrenal medulla should be inhibitory. The only explanation I could think of was that adrenal cortical secretion of substances other than cortisone in the animal was inhibitory and that cortisone administration, by inhibiting corticotrophin, had inhibited adrenal secretion of steroids other than cortisone.

Harris: I think I probably did not make the point very clear. What we imagine might be happening is that in the normal animal hypothalamic stimulation results in a sudden increase in ACTH discharge and a sudden rise in the blood concentration of adrenal cortical hormone, and that this may inhibit any increased secretion of TSH that would otherwise be elicited by the stimulation. On the other hand, if the blood

CONTRIBUTION TO THE REGULATION OF THYROID ACTIVITY

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In the Collège de France we have carried out various investigations on the regulation of thyroid activity.

To begin with, a simple and reliable procedure is required to measure this activity. We take advantage of a fact which we noticed in 1944 (Joliot, Courrier, Horeau and Sue). In the blood, a discrimination is made by the red blood cell between inorganic iodine and hormonal iodine: whereas the former is distributed between the plasma and the cells, the latter remains in the plasma. The following is our usual procedure. after the injection of radioactive iodine, a sample of blood is withdrawn and centrifuged; the radioactivity is then measured using an equal weight of red blood cells (H) and plasma (P). The ratio of the radioactivities (H/P) decreases from 0·66 to 0·10 in 24 hours, and reaches a state of equilibrium. We have observed (Courrier, 1954; Courrier, Morel, Colonge and André, 1954) that the H/P value and the thyroid activity are closely related, as can be seen from Table I showing results of experiments on rabbits.

Table 1

Time after injection of $Na^{131}I$ (rabbits)	1 *hr.*	5 *hr*	10 *hr.*	24 *hr*	48 *hr.*
H/P ratio (1 mg of each material used)	0 66	0 32	0 20	0 11	0 10

C. N. H. Long: The other point I had, however—perhaps it is not fair to talk about what kills those animals that died—but these are adrenalectomized animals on a constant dose of hormone. Such animals are [illegible]

maintenance doses for adrenalectomized animals.

Harris: With regard to the ovaries, I should have mentioned that some of our rabbits that responded had also been ovariectomized. The high doses of cortisone, of the order of 20 mg./day, were only given to the animals with intact adrenals in an attempt to blockade ACTH release—and this dose was of course kept constant throughout the control and stimulation periods of any experiment. The maintenance dose of cortisone used in the adrenalectomized rabbits was around 1·5–2·5 mg./day.

the uptake of iodine by the thyroid, as was shown by Joliot *et al.* (1945*b*). A similar conclusion has been reached for 3 : 3′ : 5-triiodo-L-thyronine (Courrier, Roche, Michel, Michel and Colonge, 1954). The results are given in Table III.

Table III

Daily treatment for 7 days (groups of 12 rats)	^{131}*I one day after end of treatment* (40 μc)	% ^{131}*I fixed in thyroid after 24 hours*	
		1st expt.	*2nd expt.*
2 μg $Na^{131}I$	+	40	44
2 μg 3 : 3′ . 5-L-T_3	+	26	21
2 μg. L-T_4	+	35	33

T_3—triiodothyronine

The daily injection of physiological doses of thyroid hormones decreases the iodine uptake, triiodo-L-thyronine being more active than L-thyroxine. The same experiments have been performed (Courrier *et al*, 1955) on hypophysectomized rats injected with TSH (U.S P.) (Table IV).

Table IV
HYPOPHYSECTOMIZED RATS (GROUPS OF 6)

Daily treatment for 7 days	^{131}*I one day after end of treatment* (25 μc)	% ^{131}*I fixed after*	
		2 hours	*24 hours*
5 μg ^{131}I	+	0 3	
5 μg ^{131}I + TSH (0 2 unit)	+	6 2	29
5 μg. L-T_3 + TSH (0 2 unit)	+	6 5	18
5 μg. L-T_4 + TSH (0 2 unit)	+	7 9	24

In the hypophysectomized rat given TSH, the administration of thyroid hormones does not further decrease the iodine

A correlation of the physiological state of rats with the H/P ratio is as follows:

Physiological state (rats)	*H/P 20 hr. after injection of Na^{131}*
Normal	0·14
Thyroidectomized	0·77
Hypophysectomized	0·64
DL-Thyroxine 5 μg /day for 9 days	0·56
Incomplete hypophysectomy:	
Before	0·10
6 days after	0·51
13 days after	0·41

This procedure has been used by White (1953) in clinical work. There is general agreement that the uptake of radioactive iodine by the thyroid gives only partial information. If iodine does not penetrate into the gland the technique is quite sufficient, but iodine may be trapped without the hormonal synthesis being possible. We have noted such a dissociation with an anti-thyroid substance (Joliot *et al.*, 1954*a*). Table II gives another example which stresses the importance of the H/P ratio, the results of a study by Psychoyos in my department of the thyroid activity in artificially blind rats.

Table II

Rats	*^{131}I uptake by the thyroid %*	*H/P*
Normal (5)	46	0·14
20 days after enucleation (10)	45	0·35

The H/P ratio shows that the enucleation has induced a state of hypothyroidism.

We have seen in Table I that the injection of thyroxine (T_4) decreases the thyroid activity*; the hormone prevents

* In 1922 we noticed, independently from Cameron, that the ingestion of a thyroidal substance would induce depression of the thyroid

specificity of the action of various iodothyronines is then demonstrated as one point.

In Table Vb are given the results obtained with triiodothyroacetic acid which does not seem to be active.

Table Vb

Product administered (groups of 12 rats)	*Duration of treatment**	*% of ^{131}I uptake after:*		
		1 hr.	*2 hr*	*4 hr.*
$Na^{131}I$	1 inj of 10 µg	5 30	7 95	16 7
3:5 3'-TRIAC	1 inj. of 10 µg.	6 60	7 20	16 8
$Na^{131}I$	2 inj. of 5 µg in 24 hr.	—	11 6	—
3·5 3'-TRIAC	2 inj of 5 µg in 24 hr	—	12 6	—
$Na^{131}I$	2 inj of 5 µg daily for 3 days	—	3 3	—
3·5 3'-TRIAC	2 inj of 5 µg daily for 3 days	—	5 7	—

* $Na^{131}I$ is administered 1 hour after the last injection of the pretreatment

The question now arises: do the thyroid substances act directly on the anterior pituitary? Do they act on the hypothalamus?

In 1956, von Euler and Holmgren reported two important experiments in rabbits According to these investigators, thyroxine acts directly on the anterior pituitary, injection of the hormone into the anterior lobe inhibits the thyroidal secretion; this effect does not result from injection of the hormone into the hypothalamus. Furthermore, although a pituitary graft into the eye does not receive any hypothalamic blood, it still has an effect, although a *diminished one*, on the thyroid hormone. This intraocular graft is able to react to the injection of thyroxine. Scow and Greer in 1955 reached the same conclusion working with the mouse

These data seem to favour a direct mode of action of

uptake by the gland after two hours. These results suggest, therefore, that the hormones are acting through the pituitary by the inhibition of TSH. However, after 24 hours there is a slight decrease in the uptake. Is this due to a direct action on the gland? This fact requires further study.

Recently (Courrier, Roche, Michel, Michel and Colonge, unpublished observations) we have studied the action of various iodothyronines on the iodine uptake by the rat thyroid. The four physiological products actually known to be secreted by the gland and circulating in the blood have relative antigoitrogenic activities of 1 for L-thyroxine, 5–7 for 3:5:3′-triiodo-L-thyronine, 0·05 for 3:3′:5′-triiodo-L-thyronine and 0·7–0·8 for 3:3′-diiodo-L-thyronine. It was of interest to discover if they would keep the same activity ratios in iodine trapping by the gland. The results of the experiment are given in Table Va.

Table Va

Daily treatment for 7 days (groups of 7 rats)	*% uptake of injected ^{131}I*	
	after 2 hours	*after 24 hours*
10 μg. $Na^{131}I$ (control)	4 10	19 3
10 μg. 3 : 3′-L-T_2	0 62	7 0
10 μg. 3 · 5 · 3′-L-T_3	0 59	6 6
10 μg. 3 : 3′ : 5′-L-T_3	0 67	8 2
10 μg. 3 : 5 · 3′-TRIAC	1 40	26 9
10 μg. L-T_4	0 95	11·9

T_2—diiodothyronine
TRIAC—triiodothyroacetic acid.

A comparison of the effects induced by the different substances is interesting; the activity of 3:3′:5′-triiodothyronine is well demonstrated. As this derivative is practically as ineffective on tadpole metamorphosis as it is on the goitrogenic action of 6-*n*-propylthiouracil, its activity on iodine uptake by the gland is very striking. The physiological

Table VI

Organ (rabbit)	*Radioactivity of 1 mg. of organ* / *Radioactivity of 1 mg. of plasma*
Median eminence	0 92
Anterior pituitary	0 69
Posterior pituitary	1 33
Kidney	1 62
Liver	0 44
Duodenum	0 62
Colon	0 33
Spleen	0 37
Thymus	0 44
Adrenal	0 44
Thyroid	0 34
Testis	0 48
Heart	0·52
Lungs	0·41
Striated muscle	0 51
Perirenal fat	0·37

the thyroid too. We have studied the H/P ratio in the rat. The results are given in Table VIa.

Table VIa

	H/P ratio
Controls (rats)	0 13
Cortisone (2 5 mg /day for 15 days)	0 25

Recently, with Roche, R and O. Michel, and Colonge, we have studied the concentrations of various thyroid substances in the rabbit. Table VII gives these new results.

Table VII

RATIO $\frac{\text{RADIOACTIVITY 1 MG ORGAN}}{\text{RADIOACTIVITY 1 MG PLASMA}}$, TWO HOURS AFTER INJECTION OF VARIOUS THYROID SUBSTANCES. THREE RABBITS IN EACH EXPERIMENT.

Organs	*TRIAC*	3 : 3′-L-T_2	3:3′:5′-L-T_3	3:5 3′-L-T_3	T_4*	Na*
Anterior pituitary	0 54	0 62	0 42	4 0	0 42	0 40
Posterior pituitary	0 67	3·7	1 7	10·8	3 2	0 43
Hypothalamus	0 07	0 31	0 15	0 75	—	—
Adrenals	0 22	0 33	0 29	1 4	0 24	0 29

* Values previously published (Courrier *et al*., 1951)

thyroxine on the anterior pituitary. But is one allowed to postulate that the pituitary, separated from the hypothalamus, is entirely deprived of any hypothalamic secretion? We face here the great problem of the hypothalamus-pituitary relationships and of the action of the neurosecretion by the portal vessels.

It is quite definite that the hypothalamus acts on the thyroid by a pituitary pathway. Hypophyseal grafts, hypothalamic lesions, and electrical stimulation of the hypothalamus have demonstrated this quite extensively. The works of Harris (1948, 1956), Harris and Jacobsohn (1952), Greer (1952), Bogdanove and Halmi (1953), and Ganong, Fredrickson and Hume (1955) are but a few of the important contributions on this subject.

The anterior part of the hypothalamus seems to act on the thyrotrophic function of the anterior pituitary.

Is it possible for us to give a contribution to this problem? In 1951, we noticed that radioactive iodine is selectively concentrated, not in the anterior lobe, but in the posterior lobe of the pituitary (Courrier, Horeau, Marois and Morel, 1951). Verifications of the hormonal nature of the radioactivity were carried out; we have already mentioned these data here. The localization is quite positive in the rabbit and in the monkey, and this fact has since been confirmed by several investigators. In 1955, Ford, Posner and Gross obtained the same results with radioactive thyroxine and radioactive triiodothyronine. After injection of the latter a high level of radioactivity is found in the posterior lobe of the pituitary and a lower one in the anterior lobe and in the diencephalon. We might add, today, that this selective concentration is given with ^{14}C-labelled cortisone. We have noticed (Courrier and Zizine, 1956) that in the rabbit there is a concentration of this hormone in the posterior pituitary and, to a lesser degree, in the median eminence. The results are given in Table VI.

Several investigators have noticed that cortisone induces a hypofunctional state, not only of the adrenal cortex but of

involvement of the posterior pituitary, the injection of thyroxine quite definitely decreased thyroid activity.

On the other hand, one might wonder if the concentration of thyroidal secretion in the posterior pituitary does not bear any connection with water and mineral metabolism. Morel and Simon (1956) have noticed that the variations in the concentration of thyroxine in the posterior pituitary were parallel to the antidiuretic function of the gland. Furthermore, Scavo, Ciampalini and Niccolai (1955) have studied the relationships of the thyroid and of the posterior pituitary with water and electrolyte metabolism

If the physiological meaning of the concentration of thyroidal substances in the hypothalamo-neurohypophyseal complex remains unexplained, one has to admit that such a concentration does undoubtedly exist.

REFERENCES

ARVY, L , FONTAINE, M , and GABE, M (1956) *C R Soc Biol , Paris.* In press

BOGDANOVE, E M , and HALMI, N S (1953) *Endocrinology,* 53, 274.

COURRIER, R. (1954) *Strasbourg med.,* 5, 55.

COURRIER, R , HOREAU, A , MAROIS, M , and MOREL, F. (1951) *C R. Acad Sci ,* 232, 776

COURRIER, R , MOREL, F., COLONGE, A , and ANDRÉ, S (1954). *C. R Acad Sci ,* 238, 423

COURRIER, R , ROCHE, J , MICHEL, O., MICHEL, R , and COLONGE, A. (1954) *C R. Soc Biol , Paris,* 148, 1144

COURRIER, R , ROCHE, J , MICHEL, O , MICHEL, R , and COLONGE, A. (1955). *C. R Soc. Biol , Paris,* 149, 307.

COURRIER, R , and ZIZINE, L. (1956). *C R Acad. Sci.,* 242, 315.

EULER, C VON, and HOLMGREN, B. (1956) *J. Physiol ,* 131, 137

FORD, D. H , POSNER, M , and GROSS, J (1955) *Anat. Rec ,* 121, 294.

GANONG, W. F., FREDRICKSON, D S , and HUME, D. M (1953). *Endocrinology,* 57, 353

GREER, M. A (1952) *J. clin. Endocrin ,* 12, 1259

HARRIS, G. W. (1948). *Physiol. Rev ,* 28, 139

[illegible]

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JOLIOT, F , COURRIER, R , HOREAU, A , and SUE, P (1945*b*). *C. R. Soc. Biol , Paris,* 139, 637.

The highest concentration takes place in the posterior pituitary. The difference between the two triiodothyronines is quite striking; the most active substance, 3 : 5 : 3′-triiodo-L-thyronine, is chiefly located in the posterior lobe.

What is the meaning of the concentration of thyroid substances in the posterior pituitary and in the median eminence? Has this concentration any connection with the *regulation of thyroid activity?*

Von Euler and Holmgrèn (1956) claim that the anterior pituitary is the only pathway for thyroxine in the regulation of thyroid activity. However, various hormones do modify the hypothalamic neurosecretion. This has also been shown for thyroxine in the chicken (Legait, 1955) and in the fish (Arvy, Fontaine and Gabe, 1956).

In our department, Baclesse and Guyon have studied this problem in the rat. The administration of thyroxine markedly decreases the neurosecretion in the paraventricular nucleus.

Another point deserves consideration. Several investigators think that the posthypophyseal hormones would be elaborated by the hypothalamus, and would be able through the portal circulation to stimulate the anterior lobe (Martini, Poli and Curri, 1956) With Zizine we have studied the influence of a posterior pituitary extract on the thyroid activity of the rat. The results are given in Table VIII

With Morel and Colonge we have, by hypothalamic lesions, induced a state of diabetes insipidus in the rat. In spite of the

Table VIII
(GROUPS OF 8 RATS)

	% *fixation of I*	*H/P ratio*
Controls	47 ± 3	0 16 ± 0 02
Post. pit. extract (0 5 i u /day for 15 days)	45 ± 4	0·15 ± 0·03
Post pit. extract (0 5 i.u./day) + T_4 (10 µg./day for 15 days)	6 ± 3	0 01 ± 0·03
Thyroxine (10 µg./day for 15 days)	8 ± 2	0 04 ± 0 01

Zizine: When the numerator is fairly constant and when the denominator is changing, you may postulate that this change is related to variations of the organic fraction.

Barker: I was just making the point that the situation here is reversed from the usual one where a tissue, say thyroid, is concentrating radioiodide.

B.-Grant: I did a few experiments last year with rabbits using the release curve as an index of thyroid activity. I injected into these rabbits, after a control period, increasing doses of Pitocin, Pitressin and Pituitrin, and until I got up to very big doses I saw no effect at all on the thyroid activity. With really large doses of Pitocin and Pituitrin (5 or 10 units three times a day) a slight inhibition was produced

Is there a correlation between the radioactive uptake and a special vascular circulation of the posterior pituitary? I do not think one could explain our results on a vascular basis, because results are not the same with different substances. triiodothyronine concentrates in the posterior pituitary of the rabbit more than thyroxine, whilst triiodoacetic acid does not concentrate

Gross: I would be rather surprised if one could explain Prof Courrier's results with the various iodinated compounds purely on a vascular basis. On the other hand, I think it is very specious to say because something is there it must be

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Legait, H. (1955). *C. R. Soc. Biol., Paris*, 149, 1016.
Martini, L., Poli, A. de, and Curri, S (1956). *Proc. Soc. exp. Biol, N.Y.*, 91, 490.
Morel, F., and Simon, C. (1956) *C. R. Acad. Sci.*, 242, 817.
Scavo, D., Ciampalini, L., and Niccolai, N. (1955). *Folia endocrin.*, 8, 271
Scow, R. O., and Greer, M. A. (1955). *Endocrinology*, 56, 590.
White, W. J. (1953). *J. Lab clin. Med.*, 41, 516.

DISCUSSION

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Zizine: We have checked that most of the activity in the organic

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Greer: I doubt that it is too important a factor, because in animals with transplanted pituitaries, the control of TSH secretion by thyroxine seemed to be just as good as in the pituitary contiguous with the median eminence.

Purves: The suggestion is that if you have a mechanism which can remove some of the thyroxine from the blood which passes over these cells, it would permit an increase in TSH secretion from them.

Harris: Prof. Courrier, have you ever examined the uptake of these compounds by the pineal gland?

Courrier: No.

Harris: As I remember it, the pineal showed a concentration of radioactive thyroxine in our experiments.

tissue

Albert: Prof Courrier showed us a slide where blinded animals had the same 24-hour iodine uptake as the normals had, but their H/P ratios were two to three times larger than the normals. Could you explain this to us?

Courrier. Iodine uptake is the first stage of thyroid function, but the H/P ratio is a better test of hormonal secretion, and one could observe a dissociation between iodine uptake and H/P ratio. Some antithyroid drugs produce such a dissociation.

Courrier: Thyroxine has perhaps a direct action upon the anterior pituitary, but it probably acts upon the hypothalamus since it modifies the neurosecretion of hypothalamic centres.

Gross: Dr. *Ford*, who is working on this, feels that these localizations might be related to neurosecretory pathways that they have been plotting in the rabbit hypothalamus

C. N. H. Long: My colleague, Dr. Maclean, for entirely different purposes was injecting labelled methionine; now it is true that you do not get this kind of concentration in the median eminence of the posterior lobe that you showed, but curiously enough you get the greatest concentration of the radioactive methionine in this general region of the hypothalamus.

Greer: This concentration in the paraventricular nucleus is very interesting, to me at least In rats with hypothalamic lesions, it seems that the area of the paraventricular nucleus is the one which has to be destroyed in order to prevent goitrogenesis from propylthiouracil. It is at least possible that thyroid hormone concentration there may not be r[illegible] to the posterior lobe neurosecretory process but to the control of [illegible]

[illegible] ments with ^{131}I-labelled acetic [illegible] and TETRAC —and [illegible] elled materials we could get [illegible] r and posterior pituitaries, however, they satu[illegible] hat a large dose in micrograms of material will not show any conc[illegible] ation.

I am not throwing this suggestion out as being of physiological significance but only to show that concentration may be a function of how much material is administered as well as of the concentrating power of the tissue you are investigating

Michel: We have used 2 µg per rabbit of nearly 2 kg.; exactly 1µg/kg radioactive TRIAC, and have not observed any significant concentration in the pituitary.

P.-Rivers: We used what we estimated as about 0 2 µg

Taurog: There is considerable species difference in the concentration of thyroxine in the posterior pituitary, as both Prof. Courrier and we have shown. We have found this concentration to occur in the dog and in the rabbit, but not in the rat, the guinea pig or the cat.

The concentration may be non-specific in the posterior pituitary, and it is not neces[illegible] this is the site where thyroxine has its effect on the pituitary [illegible] does not concentrate in the anterior pituitary, [illegible] hypothesis that thyroxine acts directly [illegible] ecessary, in other words, for a hormone to concen[illegible] s going to act.

Purves: Since it appears that the cells of the anterior lobe that secrete thyrotrophin may be inhibited directly by thyroxine, the accumulation of thyroxine in the median eminence may be a mechanism that filters out thyroxine from the blood which eventually passes through the anterior lobe and may be a factor in the regulation of TSH secretion.

homeostasis it is conceivable that this area may be the sole part of the central nervous system directly concerned with regulating anterior lobe function. However, it is becoming apparent that other parts of the limbic system modify hypothalamic and pituitary function to a considerable extent (Koikegami, Yamada and Usui, 1954; Sawyer, 1955). The extra-hypothalamic factors are undoubtedly more complex than has yet been demonstrated.

The present report will be primarily concerned with work done on the control of thyrotrophin secretion in our laboratory during recent years and only brief and incomplete reference will be made to the many excellent investigations conducted elsewhere.

Stalk Section

Considerable earlier work has been done on the effect of stalk section on regulation of thyrotrophin secretion. To some extent the experimental data are conflicting. This probably reflects, as emphasized by Harris, that in many experiments regeneration of the hypophyseal portal vessels occurred. Westman and Jacobsohn (1938) found histological evidence of thyroid hypofunction in the rat and rabbit after stalk section. Brolin (1947) noted that in the rat this operation prevented post-thyroidectomy hypertrophy and vacuolization of the pituitary basophilis. Barrnett and Greep (1951) reported that stalk section greatly diminished the increase in thyroid weight following thiouracil feeding in the rat More recently Harris (1955) and his collaborators have found that stalk section will interfere with iodine uptake and rate of release in the rabbit thyroid. Systemic injections of thyroxine are capable of further inhibiting radioiodine metabolism in such rabbits but the normal inhibition of stress on thyroidal ^{131}I loss is lacking

Hypothalamic Lesions

Anterior hypothalamic lesions in the rat have been found to abolish the thyroid hypertrophy which normally results from

THE INFLUENCE OF THE CENTRAL NERVOUS SYSTEM ON THE CONTROL OF THYROTROPHIN SECRETION

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It has become increasingly apparent that anterior pituitary secretion is not autonomously regulated but is in large part dependent upon the central nervous system. This has been best established for the gonadotrophic function of the pituitary, but in recent years evidence has been accumulating that thyrotrophin and adrenocorticotrophin secretion are also, to a large extent at least, influenced by the central nervous system.

There are many bits of evidence which point to the hypothalamus as being of great importance in pituitary regulation. (1) The anterior pituitary will not undergo morphogenesis unless Rathke's pouch is in close apposition to the hypothalamic anlage. (2) It seems well established that the hypothalamus does indeed exert a direct controlling influence over the posterior lobe of the pituitary. (3) Various experiments have also indicated that removal of the pituitary from direct contiguity with the hypothalamus, interruption of the hypophyseal stalk, destruction or stimulation of parts of the hypothalamus will considerably alter anterior pituitary secretion. The primary rôle of the hypothalamus has never been clearly established, however. It is possible that, so far as anterior pituitary regulation goes, the hypothalamus is merely a way station transmitting impulses from higher centres and thence relaying them to the anterior pituitary either by neurohumoral or some other form of transmission. Because of the demonstrated importance of the hypothalamus in maintaining

Greer and Erwin, 1956). Grossly, the pars anterior usually appears unaltered after hypothalamic lesions, even those involving considerable portions of the median eminence. Occasionally, infarcts may be produced in one or both lateral lobes. The alteration of pituitary trophic function does not depend upon the destruction of anterior lobe tissue, however.

Bogdanove and Halmi (1953) originally pointed out that there is a considerable reduction in the number of pituitary thyroidectomy cells in animals with effective hypothalamic lesions administered propylthiouracil and that there is also hypertrophy of the pars intermedia. These results have been confirmed in our laboratory (Siperstein, Greer and Erwin, unpublished data).

Hypothalamic Stimulation

Approximately 100 rats have been stimulated in the hypothalamic area, either remotely through a subcutaneously buried micro radio receiver (Greer and Riggle, 1956) or through direct leads. Concentration was focused particularly upon the size of the endocrine glands. It was hoped especially to stimu- [illegible] t of the hypothalamus [illegible] Stimulation was given [illegible] period of approximately three weeks. Definite and often quite striking behavioural changes were produced (Greer, 1955*a*) but no change in weight of any target endocrine gland resulted other than an apparently non-specific and inconstant slight adrenal hypertrophy.

Pituitary Transplants

Transplanting the pituitary from its normal site offers another means of studying the control of hypophyseal function. As with stalk section, earlier reports gave considerably conflicting data ranging from totally non-functional grafts to those which were as active as normal *in situ* glands. Because of the possibility of incompatibility reactions in some of the earlier work, a study was made in a large series of genetically

chronic thiouracil feeding (Greer, 1951, 1952; Bogdanove and Halmi, 1953). *In spite of this the thyroid : serum* iodide ratio, which is a reflection of the iodide-concentrating capacity of the thyroid, undergoes the usual tenfold increase resulting from chronic antithyroid drug administration. The hypothalamic area in the rat essential for the normal growth response of the thyroid to antithyroid drugs seems to lie in the midline between the paraventricular nucleus and the median eminence (Greer and Erwin, 1954). In a series of several hundred rats investigated by feeding propylthiouracil after making hypothalamic lesions it was found that lesions rostral, dorsal or lateral to the area between the paraventricular nuclei and the median eminence did not result in the loss of normal thyroid hypertrophy, whereas lesions within this area did. Almost invariably, effective lesions posterior to the paraventricular nuclei resulted in extensive atrophy of these nuclei. Lesions in the posterior hypothalamus were without effect. Exogenous thyrotrophin would cause normal thyroid hypertrophy in animals with effective lesions, indicating that the thyroid was still capable of responding normally (Greer, 1955*b*).

Ganong, Fredrickson and Hume (1955) have reported that lesions in the anterior median eminence of the dog will result [illegible] Radioiodine [illegible] hypothalamic [illegible]

Lesions must be of fairly good size in order to be effective in blocking thyrotrophin secretion. A number of animals with smaller lesions have been studied to localize more discretely the effective area, but in most of the animals a block of goitrogenesis was not produced.

The location of the thyrotrophin area appears to be anatomically and functionally distinct from areas controlling other pituitary trophic functions. Interference with gonadotrophin or adrenocorticotrophin secretion can also be produced with or without thyrotrophin involvement (Greer, 1952, 1953; Bogdanove and Halmi, 1953; Ganong, *et al.* 1955;

but there was hypertrophy of the thyroid epithelium and a marked increase in nuclear size—approximately equal to the nuclear hypertrophy in normal animals so treated.

No difference in pituitary function could be seen when the transplants were made from donors ranging from twelve-day-old foetuses to two-month-old adults, whether male or female pituitaries were used, or whether one-half, one, or four pituitaries were implanted. A preliminary experiment in rats in which the pituitaries were transplanted from newborn animals into their hypophysectomized mothers indicated roughly the same extent of maintenance of thyroidal radio-iodine metabolism as in mice. Von Euler and Holmgren (1956*b*) have recently reported that in hypophysectomized rabbits pituitary transplants will maintain thyroidal radio-iodine metabolism also.

A cytological study of the heterotopic pituitaries has been made by my colleague, Mrs Eleanor Siperstein (Siperstein and Greer, 1956). It was found that the neural lobe disappeared completely after the first week or so of transplantation, but that the anterior lobe and pars tuberalis grew very well. There was a considerable loss of chromophil cells as compared to normal pituitaries of the same age. After several months the transplanted pituitaries were much larger than normal glands of the same age. This growth consisted primarily of anterior lobe. Although chromophils were largely absent from the transplanted pars anterior, there was considerable cytological evidence of activity evidenced especially by an increase in the RNA content of the cytoplasm, enlargement of the cells and large negative Golgi images. Interestingly enough, the pars intermedia underwent the same type of hyperplasia as was seen following hypothalamic lesions. After the first few weeks, the intermediate lobe was larger than the intermediate lobe of normal *in situ* pituitaries of the same age. However, in later months further hypertrophy of the intermediate lobe did not seem to occur and the continued growth of the transplant was largely accomplished by pars anterior.

homogenous mice of the function of intraocular pituitary implants in hypophysectomized hosts (Greer, Scow and Grobstein, 1953; Scow and Greer, 1955). In none of the animals was the weight of any of the target organs, including the thyroid, maintained above the hypophysectomy level. The animals also lost body weight to the same extent as did hypophysectomized controls. In spite of this the thyroid gland maintained radioiodine metabolism at approximately the same level as that of normal intact mice and at a level manyfold that of hypophysectomized controls.

It was found that administering thyroxine in an amount somewhat exceeding their normal daily requirement would depress radioiodine metabolism in the implanted mice. Conversely, feeding propylthiouracil for three weeks, then stopping it for two days to allow excretion of the drug, would result in a very considerable increase in the thyroidal radioiodine metabolism of such animals. These reactions were roughly comparable to the changes seen in normal controls. No corresponding change was produced in the hypophysectomized control mice. In the implanted animals fed propylthiouracil the radioiodine metabolism per unit thyroid weight was in fact greatly increased above that of similarly treated normal controls. In spite of the surprising increase in thyroidal radioiodine metabolism in the PTU-treated implanted animals, only a very slight increase in thyroid weight was seen compared to the three- to fourfold hypertrophy produced in normal controls. Preliminary studies have indicated that although radioiodine metabolism in the mice with heterotopic pituitaries is approximately the same as that of intact mice, they are only producing one-tenth as much thyroxine daily (Greer and Scow, unpublished data).

Although the thyroid weight of the hypophysectomized, implanted mice was the same as that of hypophysectomized controls, cytologically the thyroids were somewhere between those of hypophysectomized and normal animals. With chronic propylthiouracil administration, there was only a slight increase in thyroid weight in the implanted animals;

In Vitro Studies

In view of the report by Guillemin and Rosenberg (1955) that roller tube cultures of pituitary tissue could be stimulated to elaborate ACTH upon the addition of hypothalamic tissue, a similar experiment was undertaken with mouse pituitary and hypothalamus to ascertain whether thyrotrophin secretion would be stimulated by the addition of hypothalamic tissue (Florsheim, Imagawa and Greer, unpublished). Only preliminary results have been obtained as yet, but they indicate that after the fourth day no elaboration of thyrotrophin is produced by the *in vitro* pituitaries, even following the addition of hypothalamic tissue. Assays for thyrotrophin utilized the stimulation of ^{32}P uptake in the chick thyroid (Kriss and Greenspan, 1954) and stimulation of phospholipid turnover in beef thyroid slices (Florsheim, Moskowitz and Morton, 1956).

Decortication

In an attempt to determine how much of the central nervous system could be removed without significantly interfering with thyrotrophin secretion, an attempt was made to remove the cerebrum of adult rats. It was not found possible to keep the animals alive more than a week, however, even with forced-feeding and the daily use of antibiotics. Therefore, the operation was limited to removal of the neocortex. The animals were then injected with propylthiouracil for a period of ten days after allowing one to four weeks for recovery from the operation. It was found that approximately the same degree of thyroid hypertrophy was produced in decorticate rats as in intact animals. The ovaries, adrenals and uteri were also maintained at a size comparable to those of normal controls.

Discussion

The data mentioned above indicate that anterior hypothalamic lesions or heterotopic location of the pituitary greatly reduce the stimulus for thyroid growth. Thyroidal iodine

Because of the strange intermediate lobe hypertrophy seen after both hypothalamic lesions and pituitary transplants, it was considered possible that the factor responsible for thyroidal radioiodine metabolism might be secreted by the pars intermedia. Therefore, experiments were conducted in which various parts of the pituitary were implanted, some consisting of the central part which contained pars nervosa, pars intermedia and a small amount of pars anterior, and others consisting of the lobes containing only pars anterior (Greer and Siperstein, unpublished). The results of these experiments indicated that all the stimulation of thyroidal radioiodine metabolism could be accounted for by pars anterior alone. At the same time studies conducted in collaboration with Dr. R. W. Bates (Bates, Siperstein and Greer, unpublished) of separated pars anterior, pars intermedia, and pars nervosa of rabbits and rats indicated that only the anterior lobe of these species was capable of stimulating the release of iodine from the chick thyroid.

Pituitary plus Hypothalamus Transplants

Because of the possibility that the hypothalamus itself might be independently capable of regulating pituitary secretion without the influence of other parts of the central nervous system, transplants from twelve-day to fourteen-day-old foetuses with the pituitary removed in contiguity with various parts of the central nervous system were implanted into the kidney of mice (Greer and Siperstein, unpublished). Various size implants were made ranging in size from those containing only median eminence and pituitary to those containing the whole head with the mandible and dorsum of the skull removed. Although the transplants grew well and healthy-looking nervous and pituitary tissue grew in contiguity, often with visible blood vessel connections between them, no evidence has yet been obtained of the maintenance of pituitary function beyond that seen with hypophyseal implants alone.

2·5, 3·5 or 5·0 µg. of thyroxine daily during the same period. 3·5 µg. of thyroxine has been estimated as the average daily requirement for this size rat (Dempsey and Astwood, 1943). It was found that 3·5 µg. produced a normal thyroid weight and iodide concentration. With 2·5 µg. both thyroid weight and iodide concentration were slightly increased and with 5·0 µg. daily both were slightly decreased.

Accordingly, it was postulated, as a working hypothesis, that instead of the classical one thyrotrophic hormone there might be two (Greer, 1952). One of these called "growth factor" seemed dependent upon the normal anatomical hypothalamic-pituitary relationship. The other, called "metabolic factor", seemed largely independent of the hypothalamus. It was suggested that thyroxine regulated the metabolic factor at the pituitary level since thyroid function could be increased or decreased by lowering or raising the level of circulating blood thyroxine in animals with heterotopic pituitaries. The site of thyroxine in regulating the growth factor was not apparent and it was felt that it might act either at the hypothalamic or pituitary level.

This dual thyrotrophin hypothesis has been justly criticized by Halmi (Halmi *et al.*, 1953; Halmi and Spirtos, 1954) and VanderLaan (VanderLaan and Caplan, 1954) in particular. They have pointed out, in investigations performed subsequent to the postulation of the "two thyrotrophin" hypothesis, that propylthiouracil or other antithyroid drugs cause a greater potentiation of the iodide-concentrating mechanism than they do of thyroid weight. Thyroidal iodide concentration is inversely proportional to the total thyroid iodine content. They thus believe that the results, at least those following hypothalamic lesions, are better explained by a quantitative interference with the secretion of a single thyrotrophin than by postulating two separate thyrotrophins.

They may well be correct. It is quite possible that the experiments we conducted with graded doses of propylthiouracil and large doses of propylthiouracil plus thyroxine in intact rats, which in large part led us to this belief, might

metabolism, on the other hand, seems much less interfered with by these procedures. Originally, two possible explanations suggested themselves. One was that such manipulations reduce the ability of the pituitary to secrete thyrotrophin. Thyroidal iodide concentration, however, is more sensitive than the thyroid growth response to low concentrations of thyrotropin. Thus, merely a quantitative decrease in the amount of thyrotrophin being released is produced.

The other possibility was that two separate thyrotrophic factors are secreted. One of these, causing thyroid growth, is dependent upon a normal relationship of the pituitary to an intact hypothalamus. The other, stimulating thyroidal iodine metabolism, is largely independent of the hypothalamus.

It was thought that injecting exogenous thyrotrophin in graded doses would not answer the question as to which, if either, of these hypotheses might be correct. If one assumed the possibility of two thyrotrophic factors, any increased sensitivity of one of the measured parameters to exogenous thyrotrophin might be due to greater destruction of one thyrotrophic factor than the other in the preparative fractionation of the pituitary substance. To circumvent this possibility, graded doses of propylthiouracil ranging from 0·00125 to 0·03 per cent were added to the diet of normal rats for a period of fourteen days. It was felt that the graded doses of goitrogen would produce a corresponding graded secretion of endogenous thyrotrophin. At the end of this period thyroid weights and the thyroid : serum iodide ratio were determined. Both of these parameters seemed to increase in parallel sigmoid curves (Greer, 1952). Iodide concentration was not more sensitive to small doses of propylthiouracil than was thyroid growth.

It was also possible that completely blocking doses of propylthiouracil in the presence of small amounts of thyrotrophin might produce the effect of very little thyroid growth with markedly increased thyroidal iodide concentration. This possibility was investigated by feeding a series of 100 g. rats 0·06 per cent propylthiouracil for ten days and giving them

Halmi indicate that the microscopical appearance of the thyroids of rats with effective lesions is not greatly different from that of the glands from normal untreated rats. In our laboratory, however, the rat thyroids are normally much more atrophic in appearance than they are in Iowa.

The data of Ganong, Fredrickson and Hume (1955) in the dog and our results in the rat indicate that hypothalamic lesions may also depress thyroidal iodine metabolism. Thus, if there are separate metabolic and growth factors, it is possible that the metabolic factor is not completely independent of the hypothalamus.

Apparently the neocortex is not essential to thyrotrophin release since its removal in the rat does not interfere with goitrogenesis. Earlier evidence has been presented that the neocortex is not essential to gonadotrophin production either (Davis, 1939). It appears, however, that the hypothalamus itself cannot yet be assumed to be the sole part of the central nervous system controlling thyrotrophin. Transplants of pituitary plus hypothalamus, in spite of their good growth and differentiation, do not seem more effective in maintaining endocrine function than transplants of pituitary alone.

Because alterations of the level of circulating thyroxine in animals with implanted pituitaries were capable of causing marked changes in thyrotrophin release, it was suggested that thyroxine acted directly on the pituitary itself to control the release of at least the metabolic factor from the gland. Nevertheless, another possibility is that the hypothalamus can secrete into the general circulation a substance which can influence the thyrotrophic activity of the pituitary without passing through the hypophyseal portal system. The recent results of von Euler and Holmgren (1956*a*) are much more conclusive that thyroxine does indeed act directly upon the pituitary. These investigators found that micro injections of systemically ineffective amounts of thyroxine when injected directly into the anterior pituitary would cause a decrease in the release of radioiodine from the rabbit thyroid, but that when the same amount of thyroxine was injected into the

have been different if we had first treated the animals with propylthiouracil to deplete their stores of hormonal iodine.

The results in the mice with heterotopic pituitaries are more difficult to explain with a single thyrotrophin, however. Opponents of the two thyrotrophin concept believe that the pituitary in implanted animals or in animals with hypothalamic lesions is incapable of producing more than a minimal amount of thyrotrophin. Presumably in the mice with heterotopic pituitaries this thyrotrophin already is being secreted at maximal rate since thyroid weight is not maintained above the hypophysectomy level and the production of thyroxine in these animals is considerably below that of normal controls.

This does not seem to be the case, however. Lowering the blood thyroxine level by the chronic administration of propylthiouracil or increasing it by the injection of thyroxine will result in a marked change in thyroidal radioiodine metabolism of the implanted animals and considerable cytological transformation without a corresponding alteration of thyroid size. In addition, the high level of radioiodine metabolism maintained by these animals without propylthiouracil treatment cannot be explained on the basis of a decreased content of hormonal iodine in the thyroid since the procedures of implantation and hypophysectomy would not be expected to change thyroidal iodine stores. It seems probable that the heterotopic pituitaries are not producing a normal amount of thyroxine because the hosts are essentially in a hypophysec- [illegible] less. Otherwise one would expect [illegible] ould [illegible] e [illegible]

ism in response to [illegible]

The thyroids of rats with anterior hypo[illegible] s [illegible]

[illegible] topic pituitaries. Both our results and those of Bogdanove and

GREER, M. A., and RIGGLE, G. C. (1956). *Electroenceph. clin. Neurophysiol.* In press.
GREER, M. A., SCOW, R. O., and GROBSTEIN, C. (1953). *Proc. Soc. exp. Biol., N.Y.*, 82, 28.
GUILLEMIN, R., and ROSENBERG, B (1955). *Endocrinology*, 57, 599.
HALMI, N. S., and SPIRTOS, B N. (1954). *Endocrinology*, 55, 613.
HALMI, N. S., SPIRTOS, B. N., BOGDANOVE, E. M., and LIPNER, H J. [illegible]
H [illegible]
K[illegible]
jap., 8, 7.
KRISS, J. P., and GREENSPAN, F. S. (1954) *J. clin. Endocrin. Metab.*, 14, 770.
SAWYER, C. H. (1955) *Amer. J Physiol.*, 180, 37.
SCOW, R. O, and GREER, M. A. (1955). *Endocrinology*, 56, 590.
SIPERSTEIN, E. R, and GREER, M. A. (1956) *J. nat. Cancer Inst.* In press.
VANDERLAAN, W. P., and CAPLAN, R. (1954) *Endocrinology*, 54, 437.
WESTMAN, A., and JACOBSOHN, D. (1938). *Acta path microbiol. scand.*, 15, 435.

DISCUSSION

C. N. H. Long. Miss E. G. Fry has carried out some experiments on the functional capacity of anterior pituitary tissue transplanted to the [illegible]

[illegible]

mized male rats were observed

In sixteen of the animals the adrenal weights were greater than the maximum of the controls. Further evidence of ACTH release by the transplants was shown by the fall in adrenal ascorbic acid that followed unilateral adrenalectomy and by the compensatory hypertrophy of the remaining adrenal that occurred a few weeks after unilateral adrenalectomy.

In twenty-one of the animals with transplants the thyroid weight [illegible]

In ten animals the testes re-descended into the scrotum and grew again to a size only slightly less than that of normal rats of the same age.

median eminence or other contiguous parts of the hypothalamus it was without effect.

In conclusion, it is not yet possible to state with certainty whether one or multiple thyrotrophic factors are released from the pituitary. Definite evidence is lacking either way. There is reason, however, to believe that there may be a separate metabolic and growth factor involved in the control of thyroid function. The burden of proof is always justifiably upon those who suggest a change in classical concepts of physiology. Therefore, until such time as *two* separate factors can be isolated from the pituitary, one of which has an effect only on thyroid growth and the other an effect only on thyroidal radioiodine metabolism, it is perhaps wiser to assume that only one thyrotrophic hormone exists. In the absence of this proof or of more definite physiological evidence for the existence of two such hormones this hypothesis should be seriously questioned, but may not necessarily be incorrect.

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REFERENCES

BARRNETT, R. J., and GREEP, R. O. (1951). *Amer. J. Physiol.*, 167, 569
[illegible] *crinology*, 53, 274.
[illegible] *docrinology*, 32, 509.
EULER, C. VON, and HOLMGREN, B. (1956*a*) *J. Physiol.*, 131, 125.
EULER, C. VON, and HOLMGREN, B. (1956*b*) *J. Physiol*, 131, 137.
FLORSHEIM, W. H., MOSKOWITZ, N., and MORTON, M. E. (1956). *J. clin. Endocrin. Metab.*, 16, 927.
GANONG, W. F., FREDRICKSON, D. S., and HUME, D. M. (1955). *Endo-* [illegible]
[illegible]

her normal uptake despite partial pituitary deficiency. After 48 hours

that it just re-cycles in the gland. In complete pituitary deficiency the uptake is also suppressed. This difference between complete and partial failure need not imply more than one thyrotrophin.

Harris: Dr. Long, how were the transplanted rats checked for completeness of hypophysectomy?

C. N. H. Long. We examined the sella turcica with a magnifying lens. If we see any tissue that looks at all suspicious, we section it, we do not serial section each rat.

Halmi: The maintenance of the gonad by transplants is quite sur-

trophic factors? Or is it possible that the amount of TSH required to stimulate thyroidal accumulation of ^{131}I would be smaller than the

Seven of these animals were mated and between them sired fourteen litters, leaving little doubt as to their functional capacity. In the remainder of the animals testicular regeneration did not occur.

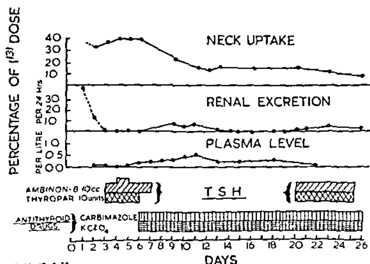
… that return of the various pituitary functions was not … ught return without … tion. In fact, almost … r pituitary activity might occur and appeared to be quite … ble from animal to

quite normal.

For contrast, let us take a second patient, whom we know had a … adenoma expanding into the cranium, and amenorrhoea … iodine

neck. So here … partial pituitary failure.

Figure 1 gives more detail of this second patient, which may explain



… same patient. Note … scharge

PITUITARY CYTOLOGY IN RELATION TO THYROTROPHIC HORMONE SECRETION

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CELLS of the group known as basophils in the pars distalis of the rat pituitary are markedly affected both by gonadal hormone deficiencies and by a deficiency of thyroid hormone. While there was a general acceptance by early investigators of the idea that the changes observed after castration were related to an increase in the output and content of the gonadotrophic hormone complex, the significance of the somewhat similar changes appearing after thyroidectomy remained for many years obscure. Our own interest in these changes resulted from our observation of similar changes accompanying the action of positive goitrogenic agents (Griesbach and Purves, 1943). The demonstration that basophil cells were specifically stimulated by even minor degrees of thyroxine deficiency showed that enlargement and an apparent increase in the number of these cells were related to an increase in the rate of thyrotrophin secretion (Griesbach and Purves, 1945).

From the investigations of Herlant (1943, 1949), Pearse (1948) and Catchpole (1947, 1949), it became clear that the characteristic cytoplasmic granules of typical basophil cells were water-soluble glycoproteins presumed to represent the content of thyrotrophin, follicle stimulating hormone (FSH) and luteinizing hormone (LH), all of which appear to be glycoprotein in character. The periodic acid-Schiff (PAS) reaction of McManus (1946), which provided a satisfactory method for the demonstration of glycoproteins in sections, greatly facilitated the further elucidation of basophil cell function.

Fraser: May not propylthiouracil increase the degree of iodine deficiency?

Human patients put on antithyroid drugs, even though still on a

activity.

functionally distinct types of basophil cell in the rat pituitary, and that the cells positively stained by it, which he termed "beta" cells, were identical with the thyrotrophs. The AF staining is due to a specific staining of the glycoprotein granules (Purves and Griesbach, 1951*b*, *c*) and indicates a chemical difference between the glycoprotein of these cells and the glycoproteins in the FSH and LH cells which collectively comprise the group termed by Halmi "delta" cells. The glycoproteins in these two cell types can be shown to be different by treatment of fresh pituitary tissue with 2·5 per cent trichloroacetic acid which precipitates the glycoprotein in the LH cells while extracting the glycoprotein from the FSH cells. This differential solubility parallels the behaviour of the respective isolated hormones (Barrnett, Ladman and McAllaster, 1955).

Herlant (1956) has shown that hibernating species of bats provide especially favourable material for the study of the specific functions of pituitary cells types. In the pars distalis of *Myotis myotis* (Borkhausen) he has demonstrated the presence of five distinct chromophil cell types. Three of these contain cytoplasmic glycoproteins. The thyrotrophs are stainable by aldehyde-fuchsin and stain blue by the Heidenhain's azan or the Cleveland-Wolfe trichrome staining methods. They are therefore similar in staining reactions to the thyrotrophs of the rat pituitary. The FSH cells, however, are also stainable by aldehyde-fuchsin and are not to be distinguished from thyrotrophs by staining affinity although they can be distinguished by morphological characters. The LH cells are not stainable by aldehyde-fuchsin and their granules are strongly acidophilic so that by the trichrome staining methods they are stained red and appear to be acidophils. It is clear that the staining reactions of the two gonadotrophic cell types in *Myotis* are entirely different from those of the corresponding functional types in the rat pituitary, and that in *Myotis* a differentiation between thyrotrophs and gonadotrophs is not obtained by aldehyde-fuchsin staining.

It is now clear that three distinct types of cell, each containing a specific and chemically distinct glycoprotein, together constitute the group of cells known as basophils in the rat pituitary. In demonstrating this, advantage was taken of the fact that treatment with thyroxine reduces the thyrotrophin content of the rat pituitary to a low level without affecting the gonadotrophin content, while treatment with oestrogen depresses the gonadotrophin content without affecting the thyrotrophin content. By this means it can be shown that the thyrotrophin is present as glycoprotein granules in cells which are angular in outline while the gonadotrophins are found in cells which are oval or round (Purves and Griesbach, 1951*a*). Treatment of adult female rats with testosterone results in pituitary glands with high FSH content and little or no LH. In such glands the glycoprotein is found in rounded cells situated in the peripheral zone of the pars distalis. On the other hand after castration, when the pituitary gland has a high luteinizing hormone content, the predominant glycoprotein-containing cell is a rounded cell dispersed throughout the interior of the pars distalis (Purves and Griesbach, 1954, 1955). The fact that each of these cell types has its own characteristic distribution indicates that they are distinct and individual cell types and not different aspects of a single cell type under altered conditions of function. We have therefore designated these types by names indicative of their function, namely thyrotrophs, FSH gonadotrophs, and LH gonadotrophs.

Romeis (1940) reported that staining of pituitary sections by the elastic tissue stains, kresofuchsin or resorcin-fuchsin, followed by azan staining enabled the differentiation of two kinds of basophil cells in a number of mammalian species. He ascribed the differential coloration of two kinds of basophils by this "Kresazan" method to the action of kresofuchsin or resorcin-fuchsin in staining some of the basophil cells and leaving others unstained. Halmi (1950; 1951*a*, *b*; 1952*a*, *b*) showed that another elastic tissue stain, Gomori's (1950) aldehyde-fuchsin (AF), produced a clear-cut separation of

thyrotrophs of the human pituitary, are similar in staining reactions to the thyrotrophs of the rat, bat, and dog pituitaries.

Herlant (1956) has indicated that he considers that the blue basophil or thyrotroph of the human pituitary is the cell which Romeis (1940) designated as the "delta" cell while the purple basophil is the cell designated by Romeis as the "beta" cell. After examination of this question we are in agreement with Herlant. The "blue" cells appear to be the only cells present to which the term "delta" could be applied, and the "purple" cells which are in preponderance in normal human pituitaries are obviously the "beta" cells. After applying the Romeis-Krezan staining method to human pituitaries we have observed the purple cells with the exact appearance of the cells described by Romeis as beta cells while the blue cells have approximately the appearance of the cells described and figured by Romeis as delta cells. Romeis, however, stated that beta cells were stained by resorcin-fuchsin while delta cells were unstained. In this particular Romeis appears to be mistaken. As in the dog pituitary, we find in the human pituitary that both the blue and the purple cells are stained either by aldehyde-fuchsin or by resorcin-fuchsin. Differentiation of these cells is in each case obtained by the added trichrome counter-stain which adds a blue tint to the blue cells and gives to the purple cells a reddish tint.

From the above it appears that while the staining reactions of the basophils of the human and dog pituitaries are similar, the usage of the terms "beta" and "delta" by Romeis in the human is the reverse of the usage of Goldberg and Chaikoff in the dog. Moreover, in neither case does the usage conform with the definition given by Romeis, and actually followed by Halmi, of delta cells as cells which are not stained by resorcin-fuchsin, or by the similar stain aldehyde-fuchsin. Such discrepancies in the application of the terms "beta" and "delta" are a potent source of confusion, particularly because of the assumption that cells similarly designated in different species are functionally equivalent. It now appears that the

In the dog pituitary Goldberg and Chaikoff (1952*a*, *b*) showed that five specific cell types could be differentiated by staining by Crossmon's (1937) modification of Mallory's trichrome stain. One cell type which stains blue by this method is specifically stimulated by thyroxine deficiency and is presumed to be responsible for thyrotrophin secretion. Goldberg and Chaikoff designated this cell as the "beta" cell; we prefer, for reasons which will appear later, to call it the "blue" basophil. A second cell type which, like the blue cell, gives a strong PAS reaction for glycoprotein, they called the "delta" cell. This cell type is more acidophilic than the blue cell and retains some acid fuchsin so that it is stained a reddish or purple shade. Our own name for this cell is the "purple" basophil. According to Goldberg and Chaikoff the blue cells were stained by aldehyde-fuchsin while the purple cells were not In our own investigations we have always found that both the blue and the purple cells are stained by aldehyde-fuchsin or by resorcin-fuchsin. The staining reactions of the purple cells of the dog, presumed to be gonadotrophic in function, are different from those of any of the cells observed in either the rat or the bat

In the human pituitary we have observed two types of basophil cell corresponding in their staining reactions to the "blue" and "purple" basophils of the dog. As Herlant (1953) has indicated, these cell types can be differentiated by counter-staining PAS-stained sections with phosphotungstic acid-orange G. The purple basophils being relatively acidophilic retain the orange G and become brick red, while the blue basophils remain a magenta colour. This differentiation is especially easy after fixation in chromate-containing fixatives which so increase the acidophilia of the "purple" cells that they stain by the azan method a red colour indistinguishable from the red colour of typical acidophils. Now Herlant (1953, 1956) has observed that the blue basophils are specially stimulated by thyroxine deficiency and give rise to the voluminous thyroxine deficiency cells seen in the human pituitary in myxoedema. These cells, which are the

GOMORI, G. (1950). *Amer. J. clin. Path.*, 20, 665.
GRIESBACH, W. E., and PURVES, H. D. (1943). *Brit. J. exp. Path*, 24, 174.
GRIESBACH, W. E., and PURVES, H. D. (1945). *Brit. J. exp. Path*, 26, 13.
HALMI, N. S. (1950). *Endocrinology*, 47, 289.
HALMI, N. S. (1951*a*). *Stain Technol.*, 27, 61.
HALMI, N. S. (1951*b*) *Anat Rec.*, 109, 300.
HALMI, N. S. (1952*a*). *Endocrinology*, 50, 140.
HALMI, N. S. (1952*b*). *Anat. Rec.*, 112, 17
HERLANT, M. (1943). *Arch. Biol., Paris*, 54, 225.
HERLANT, M. (1949). *Nature, Lond.*, 164, 703.
HERLANT, M. (1953). *II*e *Réun. Endocrin*, p 81.
HERLANT, M. (1956). *Arch. Biol., Paris*, 67, 89.
McMANUS, J. F A. (1946). *Nature, Lond*, 158, 202.
PEARSE, A. G E. (1948). *Nature, Lond.*, 162, 651.
PURVES, H. D, and GRIESBACH, W. E. (1951*a*). *Endocrinology*, 49, 244.
PURVES, H. D., and GRIESBACH, W. E. (1951*b*) *Endocrinology*, 49, 427.
PURVES, H. D., and GRIESBACH, W. E. (1951*c*). *Endocrinology*, 49, 652.
PURVES, H. D., and GRIESBACH, W. E. (1954). *Endocrinology*, 55, 785.
PURVES, H. D., and GRIESBACH, W. E. (1955). *Endocrinology*, 56, 374.
ROMEIS, B. (1940). *In* v. Mollendorff's Handbuch der mikro-skopische Anatomie des Menschen, 6, part 3. Berlin. Springer.
SMELSER, G. K. (1944). *Endocrinology*, 34, 39.

DISCUSSION

[illegible]

Halmi: There are but a few points in Dr Purves' presentation that I cannot agree with First, it is too early to conclude that what we stain in these thyrotrophs is actually thyrotrophin—perhaps we should hold back on that.

[illegible]

central in the lobe, as you have found.

[illegible]

pituitary, containing all its soluble proteins, is more potent simultaneously in FSH, LH and TSH than any preparation that was available, say two years ago. There is nothing, therefore, in the potency to suggest at the present time that these are not the "true" hormones

division of basophil cells into beta and delta cells on the basis of stainability by aldehyde-fuchsin has no consistent functional significance. Although the thyrotrophs appear, in the mammalian species so far studied, to have consistent staining properties, the staining reactions of the other glycoprotein-containing cells are so diverse that a differentiation of thyrotrophs from two types of gonadotrophs is not to be simply obtained in the generality of mammals, as it is in the rat, by a single staining procedure.

It may be pertinent to refer here to the problem of the site of corticotrophin production in the pituitary, and particularly to the question whether corticotrophin might be secreted by the same cells as are responsible for thyrotrophin secretion. In the pars distalis of the pig pituitary there is a particularly sharply delimited basophil zone situated medially and adjacent to the attachment of the stalk. This zone is entirely free from acidophil cells and can be easily distinguished in the fresh tissue. We have assayed the basophil and acidophil zones of the pig pituitary for thyrotrophin and corticotrophin, using for the latter an ascorbic acid depletion method. Thyrotrophin is strongly concentrated in the basophil zone, its concentration there being about one hundred times that of the acidophil zone. On the other hand corticotrophin is much more evenly distributed, its concentration in the basophil zone being only about twice that of the acidophil zone. This result confirms the conclusion reached by Smelser (1944), after a similar investigation of the bovine pituitary, that corticotrophin and thyrotrophin must be secreted by different cells since the hormones are differently distributed within the gland.

REFERENCES

EFFECTS OF HYPOPHYSECTOMY ON ORGANIC IODINE FORMATION IN RAT THYROIDS

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IN 1942, shortly after ^{131}I became available for biological research, Morton, Perlman, Anderson, and Chaikoff reported that the formation of ^{131}I-thyroxine by the thyroid glands of rats was greatly depressed in the absence of the pituitary, whereas ^{131}I-diiodotyrosine formation appeared to be almost normal. They concluded that hypophysectomy imposes a specific block on the conversion of diiodotyrosine to thyroxine.

Later investigators (Albert and Lorenz, 1951; Roche *et al.*, 1953) did not find so great a depression in ^{131}I-thyroxine formation by the thyroids of hypophysectomized rats as did Morton and co-workers. These later workers concluded that the absence of the pituitary does not have any specific influence on the condensation of iodotyrosines to iodothyronines in the thyroid gland.

The discrepancy between the early results in this laboratory and those of later investigators led us to reinvestigate the effect of hypophysectomy on thyroidal iodine metabolism in rats. We report here the results of some recent experiments, based on the improved techniques now available for the hydrolysis and separation of the iodine compounds in the thyroid gland.

Methods

Male rats (body weight approximately 200 g.; 45–50 days old) that had been raised on a diet consisting essentially of Purina Laboratory Chow, were hypophysectomized by the

feel about the changes in the human basophils
Crooke's hyalin change in
change

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al

Purves. I hope to ... All I can say at the p... There are cases ... Cushing's disease. All I can say at the ... There are cases ... syndrome can have different causes. There are cases ... the carcinoma ... carcinoma in which there is an autonomous secretion from the carcinoma and ACTH secretion from the pituitary is *entirely suppressed*, as shown by atrophy of the contralateral cortex and so on. There are other cases in which there is bilateral cortical hyperplasia, presumably due to an *excess* of ACTH secretion. Now, the pituitary appearances observed and described, in particular the changes in the basophil cells and the adenomas of basophil cells, are the same for both types of case, so they are unrelated to ACTH secretion

Zizine I think you mentioned that for gonadotrophic hormone you get two different kinds of cells After castration, what sort of changes do you get? Do you get changes in both cells or just in *one* type?

Purves. After castration, in the first few days there is a loss of glycoproteins and then a greatly increased number of both types of cell, both accumulate glycoproteins for the next four months as the hormone content is increased; both hyalinize, showing signet-ring changes, but ...nguished by slight differences in character of granu-
...the glycoproteins contained.
animal injected with
the castration respons
testosterone?

Purves I would say that one could ... the castration response of both types of cell with an adequate dose of testosterone.

Harris Dr. Purves, have you any information with regard to the effe... of cortisone on the thyrotrophic cells in the rat, or on the thyrotroph... cells in cases of Cushing's syndrome?

Purves Dr Halmi was one of the first to show that there is a change... thyrotrophs, under some conditions, in rats that are treated with la... doses of cortisone It could, of course, be related to an alteration of T... function. Unfortunately, in many trials, both with cortisone and ... ACTH preparations, we have produced no change either in thy... function or pituitary cytology in rats.

Albert. Thyrotrophin, as you extract it, is fairly soluble in trich... acetic acid. How does the behaviour of thyrotrophic granules i... pituitary, with respect to the solubility in trichloroacetic acid, co... to the prepared hormone?

Purves: We did not use trichloroacetic acid. All our exper... were done with buffers of different pH, and under those conditi... granules in the TSH were soluble at all pH's. I think that B... Ladman and MacAllaster have in their papers an indication t... granules of the TSH cells are also soluble in trichloroacetic acid.

of the thyroidal inorganic ^{131}I, but only a very small fraction of the gland's thyroglobulin. The inorganic ^{131}I section of the chromatogram was cut out and counted as described above. This procedure usually yielded lower results for thyroidal inorganic ^{131}I than did direct chromatography of the thyroid homogenate, possibly because the latter may result in some breakdown of ^{131}I-thyroglobulin, as suggested by Wollman and Scow (1955).

Thyrotrophic hormone (Parke, Davis and Co.) was injected in some experiments, either as a single large dose or in 5 doses spaced at 12-hour intervals. All injections were subcutaneous.

Results

Uptake of ^{131}I by thyroids of hypophysectomized rats

Fig. 1 shows the uptake of ^{131}I by the thyroids of hypophysectomized and pair-fed control rats, under the conditions

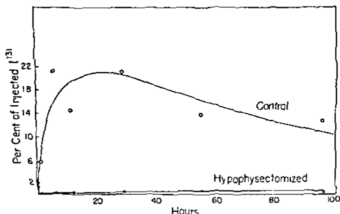


FIG. 1. Uptake of ^{131}I by thyroids of hypophysectomized and pair-fed control rats Each point represents an average value obtained with 2 or 3 rats

of our experiments. Thyroid uptake of ^{131}I was drastically reduced in rats deprived of pituitaries. At no time up to 96

parapharyngeal route. One or two days after hypophysectomy the animals were placed on a special low iodine diet (Diet 2, Taurog and Chaikoff, 1946) which maintained them in good nutritional condition. Control rats of equal weight and age were placed on the same diet. Their food intake was restricted to levels approximating to those of the operated rats.

After 5–8 days on the low iodine diet, the rats were injected with 5 μg. of iodide labelled with ^{131}I. Iodide carrier was administered for two reasons: (1) to reduce possible differences between normal and hypophysectomized rats in the concentration of circulating iodide; and (2) to accentuate differences between normal and operated rats in the capacity of their thyroids for metabolizing iodide. Groups of animals were sacrificed at intervals from 45 minutes to 96 hours after ^{131}I injection. Thyroids were homogenized, and chromatographed on filter paper, both before and after pancreatin digestion, as previously described (Taurog, Tong and Chaikoff, 1950; Taurog, Potter and Chaikoff, 1955). Plasma, too, was subjected to filter paper chromatographic analysis. Radioautograms were prepared, and the sections of the chromatograms corresponding to the various bands on the radioautograms were cut out and counted in a well-type scintillation counter. This made it possible to determine the quantitative distribution of ^{131}I in thyroids and plasma.

Thyroid/serum (T/S) radioiodide concentration ratios in
[illegible] were measured in the
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[illegible] ysed chromato-
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[illegible] determined by
the following procedure. An aliquot of the thyroid homogenate was added to 2 volumes of cold ethanol. The mixture was centrifuged at 0°, and portions of the clear supernatant were taken for chromatography. The ethanol extract contained all

blocking agent to mean that the iodide trap is the rate-limiting step in the formation of organic iodine by the thyroid. Contrary to our findings, however, Wollman and Scow also observed a decreased T/S ratio in hypophysectomized rats when organic binding of iodine was not blocked.

Distribution of ^{131}I in thyroids

(1) *Unhydrolysed glands.* In both normal and hypophysectomized rats, at all intervals measured, thyroglobulin and iodide accounted for practically all of the ^{131}I in the thyroid gland. This is illustrated in the radioautograms of Figs. 2 and 3, which show the nature of the thyroidal ^{131}I at 45 minutes and at 6 hours after ^{131}I injection. After 45 minutes a large part of the ^{131}I in the thyroids of hypophysectomized rats was still inorganic (Fig. 2) despite the very low total uptake of ^{131}I. Control thyroids at this interval, on the other hand, took up at least fifty times as much ^{131}I and converted practically all of it to thyroglobulin. There can be little doubt therefore, that hypophysectomy greatly reduced the rate of thyroglobulin formation in the thyroid gland (and also, therefore, the rate of mono- and diiodotyrosine formation). After 6 hours, the distribution of ^{131}I in the unhydrolysed thyroids of hypophysectomized rats was similar to that in control rats (Fig. 3).

(2) *Hydrolysed thyroids.* Fig. 4 shows the composition of the ^{131}I in thyroid digests from normal and hypophysectomized rats 72 hours after ^{131}I injection. A striking effect of hypophysectomy was observed on the percentage of the gland's ^{131}I present as thyroxine. Thyroxine-^{131}I comprised 16 per cent of the total thyroidal ^{131}I in control rats after 72 hours, but the corresponding figure for rats deprived of pituitary tissue was less than 1 per cent. Total iodotyrosine-^{131}I, on the other hand, constituted almost 90 per cent of the ^{131}I in the thyroids of hypophysectomized rats, but only about 60 per cent of the ^{131}I in normal thyroids. At other intervals also, as shown in Table II, the thyroids of rats without pituitaries incorporated ^{131}I into the iodotyrosine fraction at a

hours did the thyroids of the operated rats accumulate more than 0·6 per cent of the injected dose of ^{131}I. The peak uptake in the control rats, on the other hand, was about 20 per cent.

T/S Ratios in Hypophysectomized Rats

(1) *Organic binding of iodine blocked by propylthiouracil.* Table I shows typical values for the T/S ratios of hypophysectomized and control rats whose thyroids were acutely blocked by a single injection of propylthiouracil. Hypophysectomy severely reduced the ability of the thyroids to concentrate iodide—an observation reported by previous investigators (VanderLaan and Greer, 1950; Halmi *et al.*, 1953). Under the conditions of our experiments, the T/S ratio in the absence of the pituitary was approximately 2, compared with control values of almost 100.

Table I

T/S RATIOS IN NORMAL AND HYPOPHYSECTOMIZED RATS

Organic Binding of Iodine Blocked by Propylthiouracil *		*Unblocked* †	
Control	*Hypophysectomized*	*Control*	*Hypophysectomized*
89	1·7	41	1·7
96	2·0	28	1·9

* Determined 75 min after ^{131}I injection
† Determined 3 hours after ^{131}I injection

(2) *Organic binding of iodine permitted.* When no blocking agent was injected to interfere with organic iodine formation, the values for the T/S ratios in hypophysectomized rats were not significantly different from those observed in the presence of propylthiouracil. In control rats, however, T/S ratios were considerably reduced. Our findings with control rats confirm those previously reported by Wollman and Scow (1955), who interpreted the reduction in T/S ratio in the absence of a

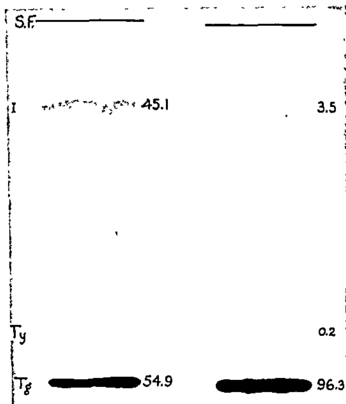


FIG 2 Radioautographs of filter paper chromatograms of unhydrolysed thyroid tissue 45 minutes after ^{131}I injection Left,

[facing page 64

Table II

Thyroxine-^{131}I and Iodotyrosine-^{131}I in Thyroid Glands of Hypophysectomized and Control Rats

Interval after ^{131}I injection	Hypophysectomized Per cent of total ^{131}I in gland		Control Per cent of total ^{131}I in gland	
hrs.	Iodotyrosine	Thyroxine	Iodotyrosine	Thyroxine
0·75	49 9	0 4	78 7	4 2
3	74 3	0 5	77 5	5 3
24	87 2	0 8	66 2	13 8

greater rate than into the thyroxine fraction. These data indicate that, under the conditions of our experiments, hypophysectomy inhibited the oxidative coupling of ^{131}I-diiodotyrosine to a much greater extent than it inhibited ^{131}I-diiodotyrosine formation.

Appearance of ^{131}I-thyroxine in plasma

Fig. 5 shows the results of chromatographic analyses of the plasmas of normal and hypophysectomized rats 70 hours after injection of ^{131}I. In the hypophysectomized rats, the thyroxine fraction of plasma contained only a minute amount of ^{131}I—less than 0·5 per cent of the total ^{131}I in plasma. It is doubtful whether this small amount of radioactivity exceeds the experimental error of the procedure. The plasma of control rats, on the other hand, contained significant amounts of ^{131}I-thyroxine even at the 3-hour interval, and in 70 hours, this fraction comprised over 80 per cent of the total plasma ^{131}I (Fig. 5).

The level of inorganic ^{131}I in the plasma decreased more slowly in hypophysectomized rats than in control rats. At the 70-hour interval, for example, almost 5 per cent of the injected ^{131}I was present as inorganic ^{131}I per 100 ml. of plasma in the operated rats, whereas the corresponding figure for control animals was 1·25 per cent (Fig. 5). These findings

S.F	0.1	0.6
I	2.8	5.3
T_3	0.1	2.5
T_4	0.9	16.1
T_m	45.6	20.2
T_y	43.7	42.1
	3.0	7.2
O	3.7	6.1

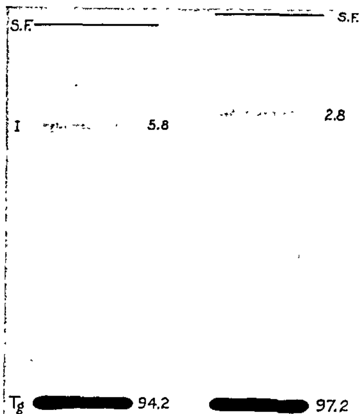


Fig 3. Same as Fig 2, except that the interval is 6 hours after ^{131}I injection.

are comparable with those reported by Morton *et al.* (1942), and may be attributed both to the decreased uptake of ^{131}I by the thyroids and the decreased renal clearance of ^{131}I (Albert, Tenney and Lorenz, 1952) which occur in the absence of the pituitary.

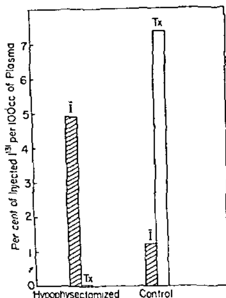


FIG. 5. Distribution of ^{131}I in plasma of normal and hypophysectomized rats 70 hours after administration of ^{131}I. Tx, thyroxine; I, inorganic iodide

Effect of administration of thyrotrophin

Injection of thyrotrophin (TSH) reversed all of the changes in thyroidal ^{131}I-metabolism induced by hypophysectomy. This is illustrated in the radioautograms shown in Fig. 6. A single large injection of TSH (Fig. 6B) produced, within 7 hours, a noticeable increase in the percentage of the gland's ^{131}I present as thyroxine. An increase in the total uptake of ^{131}I was also apparent, but there was no detectable effect on thyroid weight. The injection of 25 mg of TSH in five equal

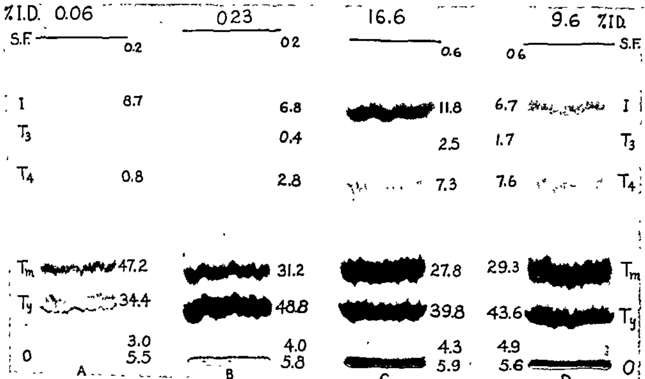


FIG 6 Radioautographs of chromatograms showing the effect of TSH injections on the distribution of ^{131}I in the hydrolysed thyroids of hypophysectomized rats 3 hours after ^{131}I injection (A) Saline-injected hypophysectomized rat (B) Hypophysectomized rat treated with a single injection of TSH (18 mg.), 4 hours before injection of ^{131}I. (C) Hypophysectomized rat treated over a period of 48 hours with 25 mg of TSH in 5 equal injections. The ^{131}I was administered 75 minutes after the last TSH injection. (D) Saline-injected control rat. The figures at the top of the radioautograph indicate the uptake of ^{131}I by the thyroids. For explanation of symbols and numbers see Fig 4.

rats in the observed T/S ratios as a result of the fact that rats which had been raised on a fairly high iodine intake were transferred, after hypophysectomy, to a diet low in iodine. At the time of the transfer, the thyroids of both control and operated rats were rich in total iodine. After the transfer, however, the thyroids of the control group came into rapid equilibrium with the new diet, and became relatively depleted of iodine. The thyroid iodine stores of the hypophysectomized rats, on the other hand, remained at the preoperative level, as indicated in Table III. If an inverse relationship exists

Table III

TOTAL THYROID IODINE IN HYPOPHYSECTOMIZED AND CONTROL RATS

Preoperative level	*Postoperative level*	
	Hypophysectomized	*Control*
mg. per cent	mg. per cent	mg. per cent
121	123	44
145	94	50
92	131	63

between the iodide trap and the iodine stores within the gland as postulated by VanderLaan and Caplan (1954) and by Halmi and Spirtos (1954), this would act to widen the difference in T/S ratios between the control and the operated rats.

The results of this investigation do not permit us to estimate quantitatively the rates of thyroid hormone production in normal and hypophysectomized rats However, if the relative rates of thyroid hormone formation are assumed to be roughly proportional to the initial rates of uptake of ^{131}I by the thyroids, then thyroid hormone formation in the hypophysectomized rats studied here was about 1 per cent of that in the controls. Randall, Lorenz and Albert (1951) measured the biological decay of ^{131}I in the thyroids of normal and

doses over a period of 48 hours restored to normal the fraction of the gland's ^{131}I present as thyroxine (Fig. 6c), while the uptake of ^{131}I and the thyroid weight were increased to values above those of the controls.

Discussion

All phases of thyroidal iodine metabolism studied in the present investigation were depressed by hypophysectomy. The iodide-concentrating capacity of the thyroid, as measured by the T/S ratio, was reduced from control values of almost 100 to values between 1·5 and 3. The rate of formation of ^{131}I-thyroglobulin was also greatly reduced in the absence of the pituitary. It is not surprising, therefore, that the total thyroid uptake of ^{131}I was depressed to extremely low levels. Formation of both ^{131}I-iodotyrosine and ^{131}I-thyroxine was greatly inhibited by hypophysectomy, but in so far as the results with ^{131}I reflect changes in the metabolism of stable iodine (^{127}I), formation of thyroxine was inhibited to a much greater extent than was that of iodotyrosine. These results confirm the finding of Morton *et al.* (1942) with respect to the striking depression of ^{131}I-thyroxine formation in the thyroids of hypophysectomized rats, but they disagree with the conclusion reached in the previous study that diiodotyrosine formation is not seriously affected by hypophysectomy.

Our failure to observe appreciable amounts of ^{131}I-thyroxine in the plasma of hypophysectomized rats even 70 hours after ^{131}I injection can be attributed to two factors: (1) the greatly diminished rate of release of thyroid hormone into the circulation (Taurog, Chaikoff and Bennett, 1946; Wolff, 1951; Randall, Lorenz and Albert, 1951), and (2) the low specific activity of the thyroxine fraction in the thyroid gland. It is doubtful whether either of these factors alone could have depressed the rate of appearance of ^{131}I-thyroxine in plasma to the low levels observed here.

The experimental conditions used here probably accentuated differences between control and hypophysectomized

thyrotrophic hormone had much more effect on organic iodine formation in the thyroid than on the iodide trap.

Our findings with the thyroids of hypophysectomized rats lead us to believe, with Stanley and Astwood, that the changes in iodine metabolism in response to alterations in the level of TSH are too widespread to be explained by a primary effect on any single phase of iodine metabolism It appears more likely that the primary effect of TSH on the thyroid is on some general and fundamental property of the tissue, such as enzyme synthesis, and that the effects on iodine metabolism are secondary to this. It is well known that TSH is essential for both the growth and maintenance of thyroid tissue Therefore, TSH undoubtedly plays an important rôle in the metabolism of thyroid proteins, and very likely in the formation of many thyroid enzymes. Iodine metabolism in the thyroid gland may be viewed as consisting of a series of consecutive reactions, beginning with the iodide trap and ending with the release of thyroid hormone into the blood. Special enzymes are very likely required at each step in the process, and the rate of formation of these enzymes may be regulated by TSH. In this manner, TSH could affect the various phases of iodine metabolism independently, and which step would be most sensitive to changes in the TSH level would depend on species characteristics and on local conditions within the gland. The fact that TSH affects such fundamental biochemical properties of thyroid tissue as O_2 consumption (VanderLaan, VanderLaan and Logan, 1941), and uptake of ^{32}P (Borell and Holmgren, 1949; Lamberg, Tala and Uotila, 1955), also suggests that it has a widespread action on the gland

Another factor in the control of thyroidal iodine metabolism by pituitary thyrotrophin, also recognized by Stanley and Astwood (1949), is that TSH regulates the iodide-concentrating capacity of the thyroid gland. The rate of any thyroid reaction involving inorganic iodine would be expected to respond to changes in the inorganic iodide concentration within the gland (simply by a mass action effect), and therefore to

hypophysectomized rats, and concluded that thyroid hormone production was reduced to about one-eighth of the normal value in animals deprived of pituitary tissue. These investigators (Randall and Albert, 1951) obtained a similar degree of depression in total thyroid uptake of ^{131}I in their hypophysectomized rats. The reason for the large discrepancy between their uptake results and ours is not apparent, but perhaps it is related to differences in the iodine intake of the animals before hypophysectomy. Their rats were placed on a Remington low iodine diet for 2–4 weeks before removal of the pituitary.

The primary site of action of TSH on iodine metabolism of the thyroid has been discussed by previous investigators (Rawson, 1949, Stanley and Astwood, 1949; Halmi *et al.*, 1953; D'Angelo, 1955). Keating and co-workers (1945) found that release of ^{131}I from the thyroids of young chicks was affected by TSH before any effect on uptake of ^{131}I could be detected. This led to the suggestion that the primary effect of TSH was on the release of thyroid hormone from the gland, and that the increased uptake of ^{131}I was a secondary effect, related to the relative iodine deficiency in the gland induced by TSH. Stanley and Astwood (1949), on the other hand, found that, in humans, a single injection of TSH enhanced the iodide-concentrating capacity of the thyroids and increased thyroidal ^{131}I uptake, but produced no measurable effect on the discharge of ^{131}I from the glands. They suggested that TSH stimulates the several components of thyroid function simultaneously. Halmi *et al.* (1953) cited results obtained by VanderLaan and Greer (1950) to support the view that the effect of TSH on the iodide-concentrating mechanism of thyroid is not secondary to its effect on iodine release. They pointed out that if hypophysectomy is performed on rats whose thyroids have been depleted of iodine by the chronic feeding of thiouracil, the T/S ratio rapidly falls from its initial high level, an effect which can hardly be attributed to changes in iodine release. The observations of Wollman and Scow (1953) with hypophysectomized mice suggest that, under the conditions of their experiments, the absence of

reduces its response to exogenous and endogenous TSH (Skanse, 1948; Maloof, Dobyns and Vickery, 1952; Doniach and Logothetopoulos, 1955; Potter, Taurog and Chaikoff, 1956). Undoubtedly, there are factors yet to be discovered which play an important rôle in modifying the action of TSH on the thyroid.

Summary

Hypophysectomy markedly depressed the following phases of ^{131}I metabolism in rat thyroids. (1) the total uptake of ^{131}I; (2) the iodide-concentrating capacity; (3) the rate of formation of ^{131}I-mono- and diiodotyrosine; (4) the rate of formation of ^{131}I-thyroxine; and (5) the rate of appearance of ^{131}I-thyroxine in plasma. ^{131}I-thyroxine formation was reduced to a much greater extent than was ^{131}I-iodotyrosine formation.

The widespread effect of hypophysectomy on the ^{131}I metabolism of rat thyroids leads us to suggest that pituitary thyrotrophin controls iodine metabolism of the thyroid in two different ways: (1) directly, by regulating, through its general metabolic effect on thyroid cells, the rate of formation of the various enzymes involved in thyroidal iodine metabolism; and (2) indirectly, by regulating the ability of the thyroid to concentrate iodide. The concentration of iodide, in turn, affects the rates of those thyroid reactions which involve inorganic iodine.

REFERENCES

ALBERT, A., and LORENZ, N. (1951). *Proc. Soc. exp. Biol., N.Y.*, 77, 204.
ALBERT, A., TENNEY, A., and LORENZ, N. (1952) *Endocrinology*, 50, 327.
BORELL, U., and HOLMGREN, H. (1949) *Acta endocr., Copenhagen*, 3, 331.
[illegible]
HARINGTON, C. R. (1951). *Endocrinology*, 49, 401
HARINGTON, C. R., and PITT-RIVERS, R. V. (1945) *Biochem. J.*, 39, 157.

changes in the level of TSH. The most obvious reactions which would be so affected are (1) the oxidation of inorganic iodide, and (2) the iodination of tyrosine residues of thyroglobulin. If, as Harington and Pitt-Rivers have postulated (Harington and Pitt-Rivers, 1945; Harington, 1951), the oxidative coupling of diiodotyrosine to form thyroxine requires iodine as an oxidizing agent, then this reaction, too, should be affected by TSH (independent of the effect of TSH on the formation of any enzymes which might be involved).

The response of thyroid tissue to exogenous or endogenous TSH is subject to modification by a variety of factors (Rawson, 1952). Excess iodide, for example, usually diminishes the stimulatory action of TSH on the thyroid, although this is not an invariable finding (Halmi, Spirtos and Stuelke, 1955). Antithyroid compounds, such as propylthiouracil, enhance the action of TSH on the gland (Halmi and Spirtos, 1954). No entirely satisfactory explanations for these effects have been offered, but the theory of TSH action discussed in the preceding paragraphs recalls the view of Wright and Trikojus (1946), and suggests that, in the presence of excess iodide, TSH is inactivated in thyroid tissue by the relatively high concentration of iodine which it, itself, helps to create. This same theory would attribute the potentiating effect of thiouracil to its ability to prevent the formation of oxidized iodine within the gland. This would supposedly minimize the rate of inactivation of TSH within the thyroid. It is of interest that the thyroid gland normally carries out a type of reaction, i.e. iodination, which is known to be capable of inactivating enzymes. This suggests that there are special mechanisms within the gland to protect it from the ill effects of excessive iodination. One of these may be the inactivation of the pituitary trophic hormone by iodine when the iodine concentration threatens to get too high.

It should be emphasized that the state of the thyroid tissue itself is also important in determining the degree of its response to TSH. For example, internal radiation with ^{131}I affects thyroid tissue in some as yet undisclosed manner which

by the work you referred to, because we felt it would be very important

done in 1942

We did these experiments with the rat in conditions apparently not very different from your present conditions. We killed the animals at different times after the administration of labelled iodide. As far as I remember, we did some experiments for 4 hours and some for 24 hours. The 4-hour results were exactly like yours. There was a relatively large excess of monoiodotyrosine present and a relatively large amount of diiodotyrosine too, but absolutely no labelled thyroxine nor triiodothyronine, which was absolutely normal.

From our findings, we formed the impression that the absence of

actually we agree.

Taurog: Yes, I think we agree that there is no qualitative effect of

a depression after hypophysectomy to approximately one-eighth of the value of the normal thyroxine secretion. I have a feeling that it is lower than that from the kind of depression of uptake we have here and from a slide which I should like to show to you when I have an opportunity.

Roche: On the question of TSH, I think you are absolutely right to

KLATING, F. R., RAWSON, R. W., PEACOCK, W., and EVANS, R. D. (1945). *Endocrinology*, 36, 137

LAMBERG, B.-A., TALA, P., and UOTILA, U. (1955) *Acta endocr., Copenhagen*, 18, 15.

MALOOF, F., DOBYNS, B. M., and VICKERY, A. L. (1952). *Endocrinology*, 50, 612.

MORTON, M. E., PERLMAN, I., ANDERSON, E., and CHAIKOFF, I. L. (1942). *Endocrinology*, 30, 495.

POTTER, G. D., TAUROG, A., and CHAIKOFF, I. L. (1956). *Endocrinology*, 59, 12

RANDALL, R. V., and ALBERT, A. (1951). *Endocrinology*, 48, 327.

RANDALL, R. V., LORENZ, N., and ALBERT, A. (1951). *Endocrinology*, 48, 339.

RAWSON, R. W. (1949) *Ann. N.Y. Acad. Sci.*, 50, 491.

RAWSON, R. W. (1952). Ciba Foundation Colloquia on Endocrinology, 4, 294. London: J. & A. Churchill, Ltd

ROCHE, J., DELTOUR, G., MICHEL, R., and VELEZ, E. (1953). *C.R. Soc. Biol., Paris*, 147, 270

SKANSE, B. N. (1948) *J. clin. Endocrin. Metab.*, 8, 707.

[illegible]

56, 122.

TAUROG, A., POTTER, G. D., and CHAIKOFF, I. L. (1955). *J. biol. Chem.*, 213, 119.

TAUROG, A., TONG, W., and CHAIKOFF, I. L. (1950) *J. biol. Chem.*, 184, 83.

VANDERLAAN, J. E., VANDERLAAN, W. P., and LOGAN, M. A. (1941). *Endocrinology*, 29, 93.

[illegible]

WRIGHT, L. E. A., and TRIKOJUS, V. M. (1946). *Med. J. Aust.*, 2, 541.

DISCUSSION

Thibault: I am very interested by this selective action of thyroxine but how do you explain the absence of triiodothyronine in the hypophysectomized rat?

Taurog: There was some indication of triiodothyronine there.

[illegible]

I don't think that is the answer myself; I think it may have more to do with the iodine content of the diet, and that is something which we are pursuing now. We did one experiment before I left in which we kept animals on a low iodine [illegible] sectomized. I expected [illegible] nearly like what you [illegible] depressed to extremely [illegible] experiments you fed Remington diet, which is lower in iodine than our diet, for two to four weeks.

When I get back, we shall have rats ready that have been on low iodine for over a month and we will see if this has any large effect.

Albert: Nevertheless, I think there is a pretty good agreement despite the fact that this experiment was done in three different laboratories.

Roche: Prof. Courrier and I agree completely with your project to work with animals kept on a low iodine diet, because we have lost a relatively large amount of time by working on animals kept on too high an iodine diet, when we studied the influence of various iodothyronines on the penetration of iodine into the thyroid gland of hypophysectomized rats.

[illegible]

of the TSH.

[illegible]

you test that animal very soon after the hypophysectomy—the time when it is usually done—before the involutionary changes occur in the gland, you obtain a maximum response.

I am not competent to say whether this is true in the case of the thyroid or not

Gross Wahlberg (1955. *Acta endocr., Copenhagen*, 20, 240) has fol-

[illegible]

think it is a sort of basic action of the thyroid cell because there is one striking thing: if one studies everything concerning the formation and release of thyroglobulin and of iodinated amino acid, one always finds a very striking depression action of hypophysectomy and a very strong stimulation action of TSH. You have that in the trapping of iodine, in the speed of the formation of iodinated amino acids, in the hydrolysis of thyroglobulin and in the release, in the deiodination processes, there is absolutely no specificity; at least I have found no sign in the literature of any known specific action.

Albert: Originally Morton and co-workers did say there was a qualitative defect after hypophysectomy. As you have just heard, we found there was a defect but it was quantitative, and the only quarrel we would have is the use of the phrase "virtual absence" of thyroxine. Now, your figures are roughly 10 per cent of what the normal rats will do in terms of thyroxine manufacture.

Taurog: They are lower than that.

Albert: I think your figure read 5 per cent for control and 0·5 per cent for hypophysectomized animals.

Taurog: But then you must realize that you have a blank to subtract; 0·5 on chromatogram like this is not to be taken as 0·5. There is probably a 0·1 or 0·2 blank because of the background on paper, which was not subtracted in this case.

Albert: Let us assume that the quantitative difference is even lower than that. Why the quantitative differences among your results, my results and Prof. Roche's? You said that the use of carrier iodide would make things more even. I wonder if that is true; would it not actually accentuate the difference in the behaviour of hypophysectomized animal versus normal?

Taurog: I said that it might even out possible differences in iodide in the circulation. This is what is initially labelled, and if one wants to infer, from the uptake, something about stable iodine it is important to initially label a pool that is of equal magnitude in the two cases.

Albert: But the hypophysectomized rat has a much lower renal clearance and a higher blood level, so that when you add carrier iodide, you are not making them the same; you are making them different.

Taurog: I do not maintain that we make the blood iodine levels the same by administering carrier, but I do think that *initial* differences in this level are reduced by using a carrier dose.

Albert: Have you a control for both the normal and the hypophysectomized animal given carrier-free ^{131}I?

Taurog: Yes.

Albert: Is it exactly the same?

Taurog: Yes. When we first started th[illegible] preliminary experiments—I cannot [illegible] many more anim[illegible] also found th[illegible] Institute of [illegible] routinely in [illegible] their thyroid uptakes are [illegible] we obtain using 5 μg of iodide carrier

Harris: Dr. Seymour Reichlin, when he was working at the Maudsley Hospital, made a study of the time relations of the responses of the rabbit's thyroid gland to the action of thyrotrophic hormone. In

the thyroid delay to the action of TSH and thyroxine.

B.-Grant: We did not really measure the delay, but what Dr. Gibson

Taurog: I was quoting you.

P.-Rivers: I know you were, but that theory was meant to explain the *in vitro* formation of thyroxine which is not an exact model.

Taurog: Well, I didn't mean to misinterpret your remarks, but it seems to me that if you think of this as an oxidative coupling which may require an oxidized form of iodine, it might happen in the intact animal as well as in your *in vitro* experiments, and I was just suggesting that as a possibility.

P.-Rivers: I like the idea of the enzyme synthesis being depressed but I do not think that iodine deficiency would work in that way.

Taurog: You mean on this particular step, or on any step?

P.-Rivers: On this particular step.

Taurog: In our laboratory, the administration of 25 μc ^{131}I to rats on a Purine Laboratory Chow diet, where the thyroid ^{131}I uptake is fairly low, produces after 30 days no detectable change—or mostly no detectable change—in the histological appearance of the thyroid. Frequently, our pathologist thinks he can see nuclear changes, but it is difficult to be sure. But the follicular structure of the thyroid is not changed, the T/S ratio is not changed, the ability of the thyroid to form organic iodine is not changed. The one thing that is changed is the

cases that you might see an effect on iodine uptake before you see an effect on release

Stanley and Astwood (1949. *Endocrinology*, 44, 49) measured in humans the effect of TSH, and in their experiments they found an effect on iodine accumulation before they were able to measure an effect on release.

B.-Grant Working with Prof. Harris on hypophysectomized rabbits, we found no evidence for a falling off in the sensitivity of the gland to TSH after operation, though I believe that when [illegible] in the department he observed [illegible] of the adrenal [illegible] tomy in th[illegible] [illegible] had an effect on the rate of release of

We certa[illegible] any effect on uptake was seen in the rabbit. However, [illegible] long be[illegible] I do not think these observations are really relevant to the question [illegible] whether TSH is acting primarily at one site [illegible]

Halmi: Dr. Long, we hav[illegible] responsive[illegible] examine[illegible] bearing [illegible] iodide p[illegible] [illegible] of the pump in my sl[illegible] [illegible] growth, so that there may be but there [illegible] difference [illegible] of the various enzymes in the cell to thyrotrophin.

Taurog: Exactly.

Fraser Have you any data, Dr Taurog, on what happens when you give an animal thyroxine and try to suppress thyrotrophin by that means rather than by hypophysectomy?

Taurog. We have not done experiments of that [illegible]

Fraser: It is a fact, I think that [illegible] vesicles, suggest [illegible]

Greer: [illegible] and the thyroi[illegible] [illegible] increased rate of release thyroxine. [illegible] to the 8-hour lag period seen when one within an h[illegible] measures thyroid uptake. The increased rate of release due to TSH persists for about 24 hours following the last dose. In contrast, the administration of thyroxine or triiodothyronine will cause as rapid an inhibition of thyroidal secretion as TSH does an increase. This seems somewhat surprising, and I should therefore like to inquire whether anyone has given TSH intravenously to human subjects to see whether the effect on secretion is gone in an hour or two or whether there is a 24-hour lag period If there is a long period during which TSH acts, even after intravenous administration, how then does thyroxine act to cut off thyroidal secretion so rapidly?

Fraser: Is it true that it does? I would not agree that thyroxine cuts off the discharge so rapidly

Greer: It has in our experience.

FACTORS INFLUENCING THE THYROIDAL IODIDE PUMP

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Introduction

THE efficiency of the thyroid as a producer of thyroid hormones is in part due to its ability to concentrate circulating iodide before incorporating it into the hormonally active compounds. The nature of the thyroidal iodide concentrating mechanism has not yet been unequivocally established. Freinkel and Ingbar (1955) have recently suggested that it involves active transport. If such is the case, the term "thyroidal iodide pump" (VanderLaan and Caplan, 1954) is justified. I shall use it throughout this paper in order to avoid more cumbrous expressions or the term "iodide trap" which manifestly ignores the fact that much of the thyroidal concentrated iodide is freely exchangeable.

Whether the transport of iodide into the thyroid is (VanderLaan, 1955; Berson and Yalow, 1955) or is not (Ingbar and Freinkel, 1956) the sole rate-controlling step in the formation of thyroid hormones, factors influencing this function can greatly affect thyroid hormone output, and thereby play an important rôle in homeostasis. In this paper some of the effects of physiological regulators and pharmacological agents on iodide pump activity will be reviewed, and special emphasis will be placed on those with which we have had personal experience.

Method

The performance of the thyroidal iodide pump is customarily assessed by determining the thyroid/serum radioiodide concentration ratio (T/S) at equilibrium, after inhibition of organic binding of iodide with a single dose of propylthiouracil

growth response of that thyroid gland to the feeding of a goitrogen, such as propylthiouracil, or to the injection of exogenous TSH; so I say that there is something about the state of that tissue which has been affected by the radiation, and very likely in the nucleus, because once in a while one can see, histologically, nuclear changes which I am not competent to evaluate, but which our pathologists think are changes.

These changes, then, affect the growth response of the thyroid to TSH. We have done considerable work on this, and so have other people. Doniach, too, has found this; and way back Skanse found the same thing. The growth response of a radiated tissue to goitrogen or to TSH is apparently one of the most sensitive indices of radiation damage that can be detected in no other way that we have at present.

Wilkinson: In your experiments have you found evidence of any biochemical lesions or irradiation artifacts?

Taurog: The biochemical changes which we measured were concerned with ^{131}I—the ability of the thyroid to form thyroxine. Perhaps if one were to investigate other aspects of the metabolism of the thyroid one might find some changes produced by the irradiation. With relatively small doses of ^{131}I-radiation we have observed no effects on the iodide trap or on the formation of organic iodine, either qualitatively or quantitatively. However, under the same conditions, there is a very definite impairment in the ability of the thyroid to show a growth response to TSH. I brought this up merely to indicate that the response of the thyroid to TSH is complex and depends on the state of the tissue, as well as on other factors.

Goolden. As regards the effect of small doses of radiation on thyroid function, I have been studying the effect of external irradiation on thyroid function in a small group of patients. These are patients with thyroid carcinoma, who have usually had carcinomatous tissue removed surgically so that the residual thyroid tissue is presumably functioning normally. I was rather surprised to find that after quite a small dose, something in the region of 500 R, there appeared to be a consistent change in the rate of release of hormone from the gland. This was inhibited for a period of about 5 or 6 days, after which there was a return to the original rate of discharge.

Taurog: This speaks again for an effect on the response to TSH after irradiation, doesn't it?

Goolden: Yes, it does

Taurog: I think we estimated the dosage to our rats at about 5000 rep. It was higher than in your experiments.

B.-Grant: Have you investigated the effect on the rate of release in the human of irradiation elsewhere in the body? Could this response you are seeing be one to the systematic effects of radiation? Thyroid inhibition as a result of whole body irradiation has been described in animal studies.

Goolden: It could well be that.

B.-Grant: Is 500R enough to produce radiation sickness?

Goolden: No.

Halmi (1954*a*) regarding the effect of graded doses of carrier iodide on the T/S at various levels of thyrotrophic stimulation, concluded that TSH does not act by increasing the *affinity* of the thyroidal iodide acceptor for its substrate. Perhaps the most plausible of the remaining possibilities is that TSH augments the *number* of iodide acceptor sites. This concept is

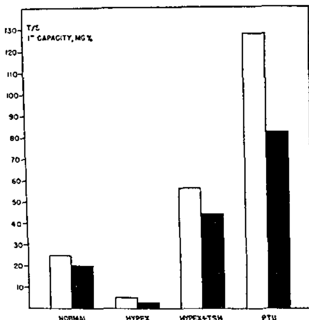


FIG 1 Effects of endogenous or exogenous TSH on the T/S (white bars) and the thyroidal capacity for pumped iodide (black bars) of rats. (cf Halmi, 1954*a*.)

in keeping with (although not proved by) the observation that the thyroid's finite capacity for pumped iodide is expanded by TSH to the same extent as the T/S for tracer doses of radioiodide is enhanced (Fig. 1) (Halmi, 1954*a*).

The level of activity of the thyroidal iodide pump is determined not only by its stimulant, TSH, but also by an intrinsic thyroidal mechanism which depresses the pump. The following findings, obtained in hypophysectomized rats, argue for

(PTU) (VanderLaan and VanderLaan, 1947; Taurog, Chaikoff and Feller, 1947). Although a gradient for iodide does exist between the unblocked thyroid and the serum of rats (Ingbar and Freinkel, 1956), acute blocking of organic binding *per se* enhances the T/S (Wollman and Scow, 1953, 1955*a*). Wollman and Scow (1955*b*) have also found that T/S may be further elevated if the decline of blood radioiodide levels is speeded up by the use of an excessively high blocking dose of PTU. The T/S value obtained with radioiodide after PTU administration therefore does not equal the stable iodide gradient between the unblocked gland and serum. However, if it is determined by a standardized procedure, it is a useful index, the variations of which in all probability parallel those of the "true" iodide gradient. Unless otherwise stated, the iodide pump studies included in this paper were carried out on PTU-blocked thyroids with tracer doses of radioiodide (method of VanderLaan and Greer, 1950).

Physiological regulators of the iodide pump

The best known regulator of the thyroidal iodide pump is thyrotrophin (TSH). The T/S of normal rats given a single dose of PTU is about 25. Enhanced output of endogenous TSH due to chronic PTU treatment may raise it to 250 or more (VanderLaan and VanderLaan, 1947; and others). Hypophysectomy lowers it substantially (Greer, 1949; and others). Exogenous TSH boosts the T/S of intact (VanderLaan and Greer, 1950) and hypophysectomized rats (Ingbar and Roberts, 1952; Halmi *et al.*, 1953). In the latter the log. dose TSH-T/S response curve is sigmoid, and may furnish the basis for an assay procedure (Halmi *et al.*, 1953). The influence of TSH on the pump is manifest in glands depleted of organic iodine-containing compounds (VanderLaan and Greer, 1950) and therefore does not appear to be secondary to enhanced thyroid hormone discharge, as suggested by Rawson (1949) However, the ultimate mode of action of TSH on thyroidal iodide transport is not understood. Wollman and Scow (1954), in analysing their own observations as well as those of

thyronine on the iodide pump of Tapazole-blocked canine thyroid slices have been unsuccessful (Fig. 2), although Molnar and collaborators (1956) could demonstrate an inhibition of ^{131}I uptake by non-blocked slices under similar experimental conditions, which suggests that the hormone does penetrate into the slices. At present the only established

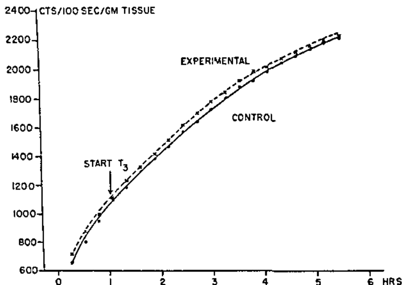


FIG. 2. Failure of L-triiodothyronine (T_3) to affect the uptake of radioiodide by canine thyroid slices blocked with Tapazole (method of Molnar *et al.*, 1956). Each curve represents the average uptake of slices incubated in two separate chambers. Abscissa: length of incubation. T_3 (20µg/100 ml of medium) added to the experimental slices 62 minutes after the start of the incubation (arrow)

effect of circulating thyroid hormones on the iodide pump is the depression which they exert by inhibiting TSH production.

Two observations apparently contradict the concepts of iodide pump regulation thus far evolved. (a) In PTU-treated rats administration of lower doses of thyroxine or triiodothyronine enhances the T/S instead of depressing it as larger doses do (Fig. 3). (b) In normal mice PTU treatment, while producing typical goitre, lowers rather than elevates the T/S.

the existence of such a depressor and delineate some of its characteristics: (a) The responsiveness of the thyroidal iodide pump to exogenous TSH is enhanced by concurrent medication with PTU, which depletes the thyroidal hormone stores (Halmi *et al*, 1953; VanderLaan and Caplan, 1954). (b) The response of the pump to a standard dose of TSH is inversely proportional to dietary iodine intake (VanderLaan and Caplan, 1954; Halmi, 1954*b*). Such is not the case if PTU is also given (Halmi and Spirtos, 1955). This shows that the effect is not due to the level of circulating iodide, but rather to products arising through organification of iodide in the thyroid. In fact, if organic binding is blocked with PTU the titres of serum iodide must rise exorbitantly (to > 100 µg. per cent) before any diminution of the T/S becomes evident (VanderLaan and VanderLaan, 1947; Halmi, 1954*a*). Moreover, this decline of the T/S is due to saturation and not to depression of the iodide pump, since the stable iodide concentration within the thyroid actually increases as the serum iodide titre rises, until the capacity level is attained (Halmi, 1954*a*). (c) Both PTU and iodine intake affect the iodide pump in hypophysectomized rats not receiving TSH (Halmi, 1954*b*; Halmi and Spirtos, 1954) This suggests that they do not merely modify the activity of TSH. (d) In our experience injected thyroxine did not diminish the iodide pump-stimulating effect of TSH (Halmi *et al.*, 1953).

All the findings listed above are consonant with the hypothesis that the pump is directly inhibited by one or more organic iodine-containing compounds within the thyroid (iodothyronines?). The activity of the pump is regulated by the interplay of TSH and the intrinsic thyroidal depressor(s) (Halmi and Spirtos, 1954, Halmi and Stuelke, 1956*b*).

We have recently re-evaluated earlier evidence for a direct action of circulating thyroid-active substances on the iodide pump of rats (Halmi *et al.*, 1953) and mice (Lipner, Wagner and Morris, 1954), and have dismissed it as inconclusive (Halmi and Stuelke, 1956*b*). Frethem, Albert and Halmi's (1956, unpublished) attempts to show an effect of triiodo-

of radioiodide in the thyroid/radioiodide in 0·1 ml. of serum" (t/S) is suggested as an index of iodide acceptor sites *per thyroid*, whereas the T/S is an index of their *concentration* in the gland. If a goitrous rat is treated with increasing doses of thyroid-active substances, the t/S at first declines less rapidly than does thyroid weight; this leads to a rise of the T/S, as a

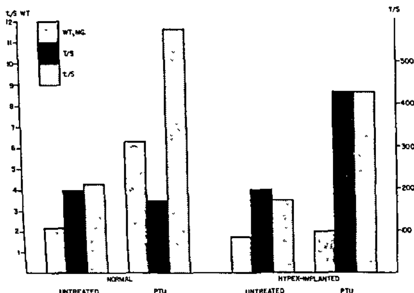


FIG 4 T/S, t/S and thyroid weight of untreated or PTU-treated normal mice and hypophysectomized mice bearing intraocular pituitary implants (Based on results of Scow and Greer, 1955.)

corollary of the resulting increase in concentration of iodide acceptor sites per unit thyroid weight (Fig. 3) In the PTU-treated intact mice the t/S rises to values which are apparently maximal for the species, but thyroid weight increases by an even greater factor. Therefore the T/S declines. In the implant-bearing hypophysectomized mice the t/S responds to PTU treatment to a somewhat lesser degree than in intact mice. However, since the weight of the gland fails to rise materially, a "dilution" of iodide acceptor sites does not occur

However, in hypophysectomized mice with intraocular pituitary implants which do not secrete enough TSH upon PTU administration to cause significant thyroid enlargement, the goitrogen brings about a marked rise in the T/S (Seow and Greer, 1955) (Fig. 4). An explanation of these paradoxes is

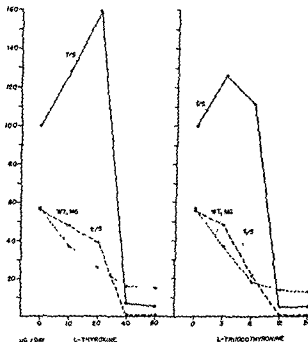


FIG. 3. Effect of graded doses of thyroxine and triiodothyronine on the T/S, t/S and thyroid weight of PTU-treated rats. PTU dosage mg/day from day 1–day 30. Thyroxine and triiodothyronine were injected in the dosages indicated from day 11–day 30. The rats were killed on day 31. (cf Halmi and Stuelke, 1956b)

possible if one makes the plausible assumptions (a) that activation of the iodide pump requires less TSH than maintenance or development of a goitre, and (b) that the number of thyroidal iodide acceptor sites per thyroid cannot be enhanced beyond a limit. (For the purposes of this discussion I consider augmentation of these sites to be the essential effect of TSH on the iodide pump.) The ratio "total amount

effect of TSH on the iodide pump is in interesting contrast with the rapid decline of TSH levels in the blood after the administration of a single dose of the hormone (D'Angelo, 1955). Even more perplexingly, we have recently found, using hypophysectomized rats made sensitive to the action of TSH on the iodide pump by either several PTU injections or prolonged feeding of a low iodide diet, that the initial effect of TSH on the T/S is one of unequivocal suppression (Fig. 5). This is true whether the hormone is injected subcutaneously or intravenously We are at present unable to explain this observation, and are planning experiments which may disclose whether the phenomenon is due to (a) "flooding" of the pump with iodide derived from the breakdown of iodotyrosines discharged from the colloid, (b) actual transient inhibition of the pump by intrathyroidal free iodothyronines liberated from thyroglobulin, or (c) temporary binding of TSH to iodide acceptor sites. Since the TSH preparations used (U.S.P. Thyrotropin Reference Substance, Armour's thyrotropin) were by no means biologically homogeneous, the possibility that the transient depression of the T/S was due to a contaminant must also be kept in mind. This uncertainty will only be resolved when pure TSH becomes available.

Pharmacological effects on the thyroidal iodide pump

The effect of certain anions on the thyroidal iodide pump is of considerable interest. It has been known for a number of years that thiocyanate interferes with the active accumulation of iodide by the thyroid and also discharges pumped iodide from the gland (Wolff *et al.*, 1946; VanderLaan and VanderLaan, 1947). Wyngaarden, Wright and Ways (1952) have shown that perchlorate is even more potent in this regard. The use of sodium perchlorate (100 mg. or more per rat) has enabled us to inhibit the iodide pump of the PTU-blocked thyroid completely and to study the iodide diffusion space of the gland. This space does not equal such anatomically defined compartments as the stromal space, the water space of stroma plus cells, or the total water space of the thyroid

and the T/S rises (Fig. 4). It is clear that changes in thyroid weight may render the T/S an unreliable index of iodide pump activity. If the t/S is used as the index, both paradoxes discussed fall in line with the previously presented fundamental scheme of iodide pump regulation.

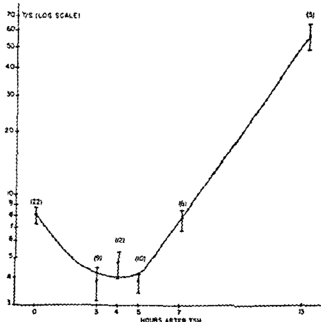


FIG. 5 Initial depression and subsequent rise of the T/S in hypophysectomized rats given a single subcutaneous injection of TSH (0 9 U.S P unit). The rats were fed a low iodine diet (Remington) for 15–19 days, starting 5 days after the operation Number of rats per group in parentheses. Intervals indicated are between injection of TSH and sacrifice Vertical lines. standard errors

It has been reported that a single subcutaneous injection of TSH does not raise the T/S of hypophysectomized rats for at least 6 hours, and that the maximal effect of a dose of TSH on the iodide pump does not become manifest until 1–2 days after the injection, whether hypophysectomized (Halmi *et al.*, 1953) or normal (VanderLaan and Greer, 1950) rats are used. This also holds true if TSH is given intravenously. The sluggish

fraction did not occur between 4 and 24 hours after the injection of the tracer (Fig. 8), which means that the concomitant rise in the T/S must have been due to falling blood radioiodide levels. The ^{131}I taken up by the iodide pump of PTU-blocked thyroids appears to be in at least two forms: freely exchangeable, perchlorate-dischargeable radioiodide,

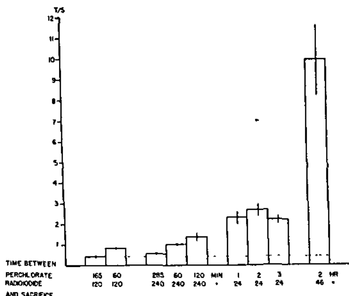
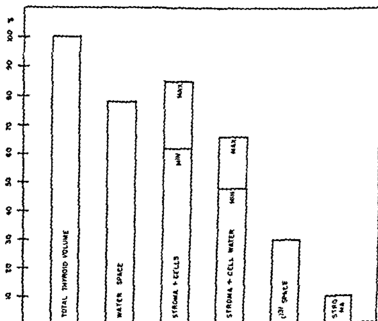


FIG 7 [illegible]
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which is in equilibrium with serum radioiodide, and a more inert fraction, which is neither in equilibrium with serum radioiodide nor dischargeable with perchlorate. Preliminary studies regarding the chemical nature of the latter fraction have been performed by Dr. Jack Gross. We sent him saline homogenates of rat thyroids in which organic binding had been blocked with chronic PTU pretreatment, and which were obtained at necropsy 24 hours after ^{131}I administration. The

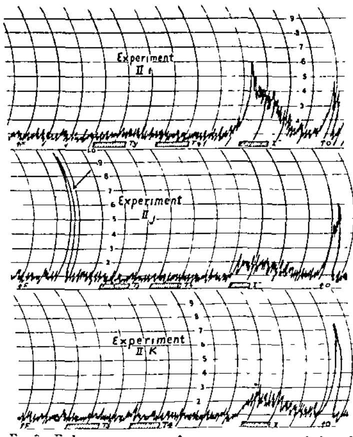
(Fig. 6). It is comparable in magnitude with the iodide space of some other organs (e.g., salivary glands, spleen) which are not affected by perchlorate. TSH has no effect on the thyroidal iodide diffusion space (Halmi, Stuelke and Schnell, 1956).

We have also found that even 200 mg. of sodium perchlorate



fails to discharge all pumped iodide from the thyroids of PTU-treated rats (Halmi and Stuelke, 1956*a*). If the perchlorate was given after ^{131}I, the T/S was never as low as when its administration preceded that of ^{131}I. The concentration ratio "non-dischargeable thyroidal ^{131}I/serum ^{131}I" increased with the length of stay of ^{131}I in the thyroid (Fig. 7), although an actual accumulation of ^{131}I in the non-dischargeable

apparently arose under the effect of perchlorate showed little electrophoretic mobility in barbital buffer at pH 8·6, and



dioxan-ammonia solvent front. The peak between F and T_3 in Experiment II J (arrow) is an artifact (By courtesy of Dr. Jack Gross)

thereby differed from rat thyroglobulin-^{131}I (Robbins and Rall, 1952). Elucidation of the chemical nature of this fraction requires further studies.

three groups of animals were given perchlorate 1 hour (Exp II i), 2 hours (Exp. II j) and 3 hours (Exp. II k) before sacrifice, respectively. The T/S was not significantly different in the three groups (cf. Fig. 7). In the chromatograms (Fig 9) two peaks of radioactivity were found: one in the region of the

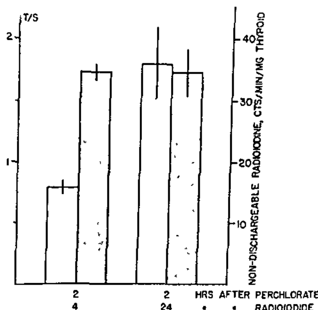


FIG. 8 Failure of the concentration of ^{131}I not discharged by perchl … while the T/S … (white bars) rise … lorate injections … with PTU). T … s. (cf

iodide carrier and one at the origin. The latter peak increased at the expense of the former *pari passu* with the duration of perchlorate action. Apparently perchlorate in some manner brought about protein-binding of the ^{131}I fraction which it failed to discharge. *Prior to the application of perchlorate the* non-dischargeable ^{131}I may have been present entirely as iodide contained in a second iodide compartment, but this idea remains to be proved. The protein-bound ^{131}I which

than the thyroid, such as salivary glands, stomach and mammary glands (Honour, Myant and Rowlands, 1952). The existence of a salivary iodide pump in the rat is doubtful (Halmi, Stuelke and Schnell, 1956). The active gastric iodide pump of this species is similar to its thyroidal counterpart in so far as it too is inhibited by perchlorate (Halmi, Stuelke and Schnell, 1956), but in hypophysectomized animals the gastric iodide pump failed to respond consistently and significantly to TSH doses which elevated the T/S tenfold or to combined PTU-TSH treatment which raised the T/S by a factor of 40 (Fig. 10). Brown (1956) has also found that gastric clearance of iodide is not affected by TSH.

Acknowledgements

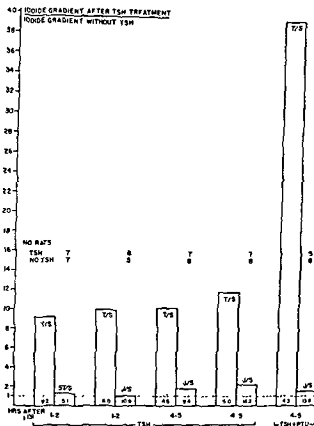
Personal experiments summarized in this paper were performed in collaboration with Drs H J. Lipner, E. M Bogdanove and B. N. [illegible]

REFERENCES

BERSON, S. A., and YALOW, R. S (1955) *J clin Invest*, 34, 186.
BROWN, J. (1956) *Endocrinology*, 58, 68
D'ANGELO, S. A. (1955) Brookhaven Symposia in Biology, No. 7, p 9.
FREINKEL, N., and INGBAR, S. H. (1955). *J. clin Endocrin. Metab.*, 15, 442
[illegible]
HALMI, N. S., and STUELKE, R G (1956b). *Metabolism*, Symposium on the thyroid, November.
HALMI, N. S, STUELKE, R. G, and SCHNELL, M. D (1956) *Endocrinology*, 58, 634.
HONOUR, A. J, MYANT, N B, and ROWLANDS, E N (1952). *Clin. Sci.*, 11, 449.
INGBAR, S. H., and FREINKEL, N (1956). *Endocrinology*, 58, 95.
INGBAR, S. H., and ROBERTS, J. (1952). *J. clin. Endocrin. Metab*, 12, 975.

Comparison of gastric and thyroidal iodide pumps

The occurrence of iodide transport against an electrochemical gradient has been observed in several organs other



the animals with transplanted pituitaries than in normal animals similarly treated. Such an explanation seems perfectly reasonable.

However, I do not feel this has much bearing on the question as to whether there are one or two thyrotrophic hormones. The difference

heterotopic pituitaries than it is in hypophysectomized mice. It is therefore quite unlikely that the iodide-concentrating mechanism is the rate-limiting process here. I do not believe that the evidence as yet permits us to explain the thyroidal effects of heterotopic pituitaries on the basis of differential sensitivities of certain processes to a single thyrotrophin, although this may ultimately prove to be the case.

Halmi: I have confined myself to the iodide pump, but have not suggested that the concentrating of iodide is necessarily the rate-limiting step in thyroid hormone formation. It definitely does not appear to be that in the rat and the mouse. I believe that Dr Greer's findings of an almost intact iodine-binding capacity of the thyroids in the mice with implanted pituitaries may indicate that steps of thyroid

such a concept comes to the fore.

Pochin: On this question of iodine which is not dischargeable by perchlorate, it would be very nice if you could find a species in which

would do.

Taurog: Has anyone investigated the dog for salivary ^{131}I concentration? I think the dog is quite easy to get saliva from, and it would be

LIPNER, H. J., WAGNER, B. P., and MORRIS, H. P. (1954). *Proc. Soc. exp. Biol., N.Y.*, 87, 578.
MOLNAR, G. D., KEATING, F. R., Jr., ORVIS, A. L., and ALBERT, A. (1956). *Endocrinology*, 58, 501.
RAWSON, R. W. (1949). *Ann. N.Y. Acad. Sci.*, 50, 491.
ROBBINS, J., and RALL, J. E. (1952). *Proc. Soc. exp. Biol., N.Y.*, 81, 530.
SCOW, R. O., and GREER, M. A. (1955) *Endocrinology*, 56, 590.
TAUROG, A., CHAIKOFF, I. L., and FELLER, D. D. (1947). *J. biol. Chem.*, 171, 189.
VANDERLAAN, J. E., and VANDERLAAN, W. P. (1947). *Endocrinology*, 40, 403.
VANDERLAAN, W. P. (1955) Brookhaven Symposia in Biology, No. 7, p. 30.
VANDERLAAN, W. P., and CAPLAN, R. (1954). *Endocrinology*, 54, 437.
VANDERLAAN, W. P., and GREER, M. A. (1950). *Endocrinology*, 47, 36.
WOLFF, J., CHAIKOFF, I. L., TAUROG, A., and RUBIN, L. (1946). *Endocrinology*, 39, 140.
WOLLMAN, S. H., and SCOW, R. O. (1953). *Endocrinology*, 53, 332.
WOLLMAN, S. H., and SCOW, R. O (1954). *Endocrinology*, 55, 828.
WOLLMAN, S. H., and SCOW, R. O. (1955*a*). *Endocrinology*, 56, 445.
WOLLMAN, S. H., and SCOW, R. O. (1955*b*). *Endocrinology*, 56, 448.
WYNGAARDEN, J. B., WRIGHT, B. M., and WAYS, P. (1952). *Endocrinology*, 50, 537.

DISCUSSION

Taurog: I am interested in this material that remained at the origin of these chromatograms and I wonder what the nature of the material is Do you think it is a protein-bound iodide?

Halmi. We don't know.

[illegible] obtained [illegible] nic— [illegible]

Halmi. It is very unlikely but you always have to think of that— [illegible]

THE "FEED-BACK" HYPOTHESIS OF THE CONTROL OF THYROID FUNCTION

K. Brown-Grant

Chemical Defence Experimental Establishment, Porton, Wiltshire

Possibly the earliest indication of the existence of a functional relationship between the thyroid and the adenohypophysis was an account of pituitary enlargement in a series of goitrous cretins (Nièpce, 1851). Since then a multitude of studies have provided detailed evidence of this relationship; D'Angelo (1955) has recently reviewed the development and present status of this concept. In the absence of the pituitary thyroid activity falls to very low levels and the gland appears to possess, at best, only minimal powers of autonomous regulation in response to such stimuli as iodine deficiency (Chapman, 1941; Goldberg, Greep and Nay, 1953) or cold (Wolf and Greep, 1937). The value to the organism as a whole of such changes as are described remains unestablished Other extra-pituitary regulating systems have been proposed. The plasma iodide level or more probably the intra-thyroidal iodide level has been shown to influence both the iodide-trapping mechanism and the organic binding of iodine in normal and hypophysectomized animals (VanderLaan, 1955; VanderLaan and Storrie, 1955). The administration of thyroxine has been claimed to decrease and goitrogenic drugs of the thiouracil type have been shown to potentiate some of the effects of exogenous TSH in hypophysectomized animals (Cortell and Rawson, 1944; Halmi and Spirtos, 1954). It has also been suggested that the rate of peripheral extra-thyroidal destruction of TSH may modify thyroid activity (Gyllensten, 1953; D'Angelo, 1955). These observations are of great interest and have led to the acquisition of much detailed

Pochin: In man, Rowlands and others have investigated and failed to find any change, using injected TSH, in the concentration ratio

materials?

Halmi: We have not but perhaps if Dr. Albert will find somebody to run the gravity flow incubator for him, we shall!

Albert: Diiodotyrosine has been tried; it is ineffective in the thyroid slice. Thyroglobulin has been tried and it, too, was ineffective. So far, nothing that we have tried either singly or in collaboration with Dr. Halmi has inhibited the thyroidal iodide pump.

Barker. Perhaps Robbins and Rall's polymer of monoiodotyrosine may play a rôle; they first found it in serum from ^{131}I-treated thyroid carcinoma patients and then in the gland itself. The existence of a polymerized form would not be ruled out by the lack of response to the free amino acids because the gland simply does not take them up.

Halmi: There is no evidence for the chemistry of the inhibitor whatsoever. But the ineffectiveness of the exogenously administered free amino acids doesn't mean that endogenous free amino acids are not the inhibitor.

Barker: But that is the point—the gland does not seem to take up free amino acids.

Halmi: Under the conditions of the slice experiment, Dr. Albert and his collaborators have found that if you do not block the slice with propylthiouracil you do get an inhibition of ^{131}I uptake with thyroglobulin and thyroxine and triiodothyronine. I hope this will be repeated with tribromothyronine where you cannot make the objection that you are introducing iodide

P.-Rivers: Is it perfused thyroxine?

Halmi: Yes, drip perfused.

P.-Rivers: How long does it take for a complete change of the thyroxine solution in the compartment?

One can regulate the flow at any rate one desires. Most of the

more markedly with small doses of thyroxine or triiodothy-

Halmi: Yes.

Greer: How many animals did you have in this experiment?

Halmi: Seven to ten animals per group.

led many workers to abandon this and other unitary theories of the control of ACTH secretion and adrenocortical activity (Harris and Fortier, 1954; Harris, 1955*a*). The validity of a similar "feed-back" concept as applied to the thyroid will be examined in the light of recent studies.

Changes in the thyroid induced by a deficiency or an excess of thyroid hormone

(1) *Deficiency of thyroid hormone*

When the synthesis of hormone is prevented by the administration of goitrogenic drugs the result, as is well established, is an increased secretion of TSH by the pituitary and a series of striking changes in the thyroid ensues. The sequence of events can be readily interpreted in terms of the "feed-back" hypothesis. D'Angelo (1955) presents the evidence on this point, though omitting the elegant studies of Dempsey and Astwood (1943) These workers showed that higher doses of thyroxine were necessary to suppress goitre formation under conditions of increased peripheral utilization brought about by exposure to reduced environmental temperature. The effects of partial thyroidectomy have recently been re-examined in the rat by Logothetopolous and Doniach (1955). While they report no unexpected findings, they have confirmed the older work and added additional quantitative data on the changes induced. Their results are consistent with the hypothesis of control discussed here.

(2) *Excess of thyroid hormone*

The effects of exogenous thyroxine have been investigated in detail in the rabbit using the rate of release of ^{131}I-labelled thyroid hormone from the gland of the conscious animal as an index of the level of thyroid activity The technique employed has been fully described by Brown-Grant, von Euler, Harris and Reichlin (1954) A single injection of 100 μg of sodium L-thyroxine resulted in a prompt and complete inhibition of

information about certain limited aspects of thyroid function. It has yet to be shown, however, that any of these mechanisms play any significant part in the regulation of thyroid activity in the normal animal. In the present paper, therefore, attention will be concentrated on the interplay of the thyroid and the pituitary as the major homeostatic mechanism for the thyroid.

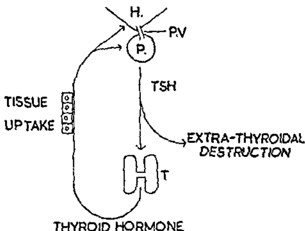


FIG 1. The conventional "feed-back" mechanism. H.—hypothalamus P.—anterior pituitary. P.V.—portal vessels TSH—thyrotrophic hormone T.—thyroid.

Early observations of the atrophy produced by the administration of thyroid hormone and of the compensatory hypertrophy after partial thyroidectomy, accompanied by changes in the blood and urinary levels of TSH, led to the formulation [illegible]

attempt to maintain. a constant level of thyroid hormone in *the blood* (Hoskins, 1949). The essence of such a system is illustrated in Fig. 1. Similar schemes have been suggested for the control of other endocrine glands, notably for the adrenal cortex (Sayers, 1950). Subsequent work, however, has

methylthiouracil gave the same value for the delay in onset of inhibition (Reichlin, 1954). The delay between the intravenous injection of TSH and the onset of an increased rate of release from the rabbit thyroid has been established as about one hour (Brown-Grant *et al.*, 1954; Reichlin and Reid, 1955); allowing for the time involved in the absorption of thyroxine after subcutaneous or intraperitoneal injection, it will be seen that the pituitary reaction to a raised blood level of thyroxine is far more rapid than had been suspected, as indeed is the speed of response of the thyroid itself.

Apart from the rapidity of reaction of the thyroid-pituitary system the second finding of interest concerned the sensitivity of this system. The release of ^{131}I from the thyroid was determined over an initial control period during which daily injections of 0·005N-NaOH were given. The effect of daily injections of thyroxine or triiodothyronine in 0·005N-NaOH was then observed. Beginning with a dose of about 12 µg./day a slowing in the rate of release was observed; as the dose was increased by 1 or 2 µg./day, the rate of release was reduced still further until at a dose of about 16 µg./day complete inhibition was produced. Reduction of 1 or 2 µg./day permitted the release of ^{131}I from the thyroid to begin again after a delay of about 24 hours, and increasing the dose again by the small amount resulted in a second period of complete inhibition (Brown-Grant, 1955*b*).

Both the speed of action and the sensitivity of the thyroid-pituitary axis in the rabbit were demonstrated in these experiments using the rate of release of ^{131}I from the thyroid as a measure of thyroid activity. It seems doubtful if these observations could have been made using any of the techniques for measuring the uptake of ^{131}I that have been described. The relative sluggishness of changes in uptake has been mentioned previously in connection with the effect of thyroxine; as a further example the effect of hypophysectomy may be quoted. Whereas the release of ^{131}I falls to very low rates within 4–8 hours of operation in the rabbit (Brown-Grant *et al.*, 1954), [illegible] land only falls

the release of radioiodine from the thyroid, lasting for 50 to 100 hours (Fig. 2). The use of the "release-curve" method enabled certain aspects of the "feed-back" mechanism to be more clearly appreciated than had previously been possible. The delay between the injection of thyroxine and the onset of inhibition was determined by extrapolating the line through the points obtained during the period of inhibition and the preceding control period. In nearly two-thirds of a series of

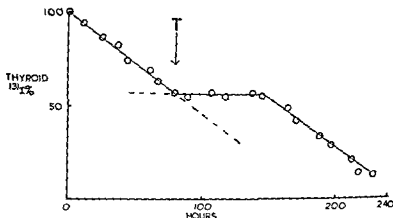


Fig. 2. The effect of thyroxine (80 μg injected subcutaneously at T) on the rate of release of thyroidal ^{131}I in the rabbit 0 hr —48 hr. after injection of 3 μc of ^{131}I. (*From data of Brown-Grant, 1955a.*)

nineteen experiments, the delay was found to be less than two hours. Although the reaccumulation of ^{131}I from degraded thyroxine was found to be only 10 per cent in rabbits under the conditions of these experiments (Brown-Grant, von Euler, Harris and Reichlin, 1954), the observation that the uptake of ^{131}I by the rabbit thyroid is only slowly affected by variations in the level of TSH stimulation (Brown-Grant and Gibson, 1955) raised the question of whether continued uptake of ^{131}I after release from the gland had ceased might vitiate this estimate of the delay in onset of inhibition. However, experiments performed on rabbits in which reaccumulation of ^{131}I by the thyroid had been blocked by the administration of

also described a slight but probably significant *decrease* in the PBI of intact cold exposed rats at a time when thyroid activity was unequivocally increased, thus confirming the findings of Ershoff and Golub (1951). Similarly, Gottschalk and Riggs (1952) report a slight fall in the PBI of a small group of American soldiers in the Arctic, while Stevens, D'Angelo, Paschkis, Cantarow and Sunderman (1955) found no change or a slight fall in cold-exposed guinea pigs in which the BMR, thyroid weight and the rate of release of ^{131}I from the thyroid were markedly increased. Thus an increased rate of peripheral utilization resulting in a lowered blood level of hormone, may act via the "feed-back" mechanism to produce increased thyroid activity in the cold. More information is desirable, however, on the rate at which this fall in peripheral PBI develops and also as to its magnitude. However, both the sensitivity of the system and other factors to be discussed later should be considered before alternative explanations are sought for the changes observed in the cold.

Changes in thyroid activity induced by other experimental procedures

In this section the effects of emotional and physical stress and of high doses of cortisone or stilboestrol on the thyroid function of rabbits will be discussed

Earlier work had shown that the above procedures led to a reduction in the rate of release of thyroidal radioiodine (Brown-Grant, Harris and Reichlin, 1954*a*, *b*) and subsequently it was shown that they also produced a decrease in the iodide clearance rate of the thyroid (Brown-Grant and Gibson, 1955; Gibson, Harris and Skynner, 1954). On the basis of these observations and the information available in the literature, which is summarized in the above papers and by Harris (1955*a*), it seemed probable that the mechanism of these changes was a depression of pituitary TSH secretion. It was therefore of some interest to know whether these changes could be interpreted in terms of the conventional "feed-back"

to its final level after some 10 to 14 days (Brown-Grant and Gibson, 1955).

(3) *The effect of cold*

It is unfortunate, therefore, in view of the rapidity with which changes in thyroid activity have been shown to follow increases in the level of circulating hormone that the majority of studies on the reaction of the gland to cold have been relatively long term experiments. Such acute studies as have been made were for the most part concerned with the response of the thyroid to extreme degrees of cold (about 0° C), and both in the rabbit and in the rat, these very low temperatures have been shown to be a less effective stimulus to the thyroid than moderate degrees of cold. This may be because severe cold is an effective stimulus of ACTH release. A fuller discussion of this point will be found in Harris (1955*a*, *b*) and Brown-Grant (1956).

The available evidence, however, gives some support to the thesis that a decrease in the level of circulating hormone may be the proximal cause of increased TSH secretion and thyroid activation in the cold. The time relationships involved—an increased rate of release from the thyroid is observed after 4–8 hours cold exposure in the rabbit and the rat (Brown-Grant, 1956)—are within the limits set by the speed at which the pituitary is known to be capable of responding. An increased rate of peripheral utilization or disposal of thyroid hormone is well established (Dempsey and Astwood, 1943). Bondy and Hagewood (1952) showed that a marked fall in stable (^{127}I) protein-bound iodine was established 15 hours after cold exposure in intact rats; their additional observation that an even more marked fall was observed in thyroidectomized rats maintained on constant doses of thyroxine shows that this is due to an increased rate of peripheral utilization. The studies of Rand, Riggs and Talbot (1952) confirmed the increased rate of utilization of hormone in cold-exposed rats and the increased daily dosage needed to maintain normal concentrations in thiouracil-treated animals in the cold. They

the total activity was in this fraction. The highest absolute concentration was usually observed between 48 and 60 hours after injection, that is between 12 and 24 hours after the time of peak thyroid content (Fig. 3). From this point on, the $PB^{131}I$ level, after correction for isotope decay, declined exponentially with a half time constant that appeared to be

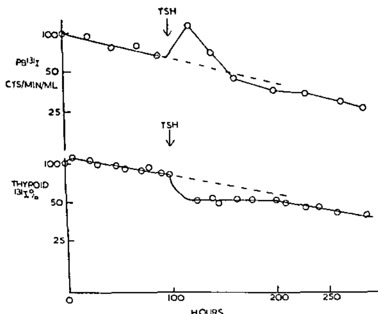


FIG. 4 The effect of TSH (2 mg U S P equivalent subcutaneously at ↓) on the rate of release of thyroidal radioiodine and $PB^{131}I$ levels (From Brown-Grant, 1955*a*)

identical, within the limits of experimental error, with that of thyroidal radioiodine.

TSH administration (2 mg. U.S.P. equivalent subcutaneously) produced a marked rise in $PB^{131}I$ above the predicted level; following the rapid discharge of radioiodine from the thyroid, the rate of release fell to almost zero and this period of "compensatory inhibition" persisted until the elevated $PB^{131}I$ level fell to or below the predicted level (Fig. 4). This

hypothesis, and experiments designed to permit the simultaneous measurement of the blood level of hormone and the activity of the thyroid were performed.

(1) *The technique and its application*

Full details of the method may be found elsewhere (Brown-Grant, 1955*a*). Briefly, rabbits were injected with a large

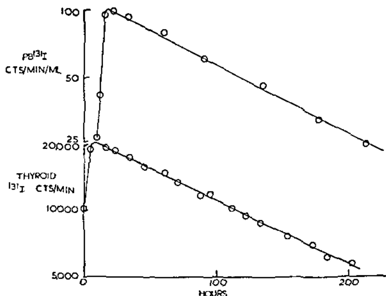


FIG. 3 To show the parallelism of the thyroid ^{131}I content and the $PB^{131}I$ level of the peripheral blood 0 hr.–18 hr. after the injection of 40μc of ^{131}I (From Brown-Grant, 1955*a*)

(40 μc) dose of carrier-free radioiodine and the rate of release of ^{131}I from the thyroid studied by *in vivo* counting for ten to twelve days, beginning 48 hours after injection. Daily measurements were made of the plasma $PB^{131}I$ (trichloracetic acid precipitable radioactivity). In preliminary experiments, a significant proportion (about 5 per cent) of the total plasma radioactivity was found in the $PB^{131}I$ at about 4 hours after injection. From 30 hours onwards more than 90 per cent of

causing a rise in blood hormone levels and consequent inhibition of TSH secretion.

(3) *The effect of cortisone and stilboestrol*

Injection of cortisone (5 mg. twice daily for three days) or stilboestrol (5 mg.) produced the expected complete temporary inhibition of release (Fig. 6) In both instances this was associated with a fall in $PB^{131}I$ levels followed by a return to normal

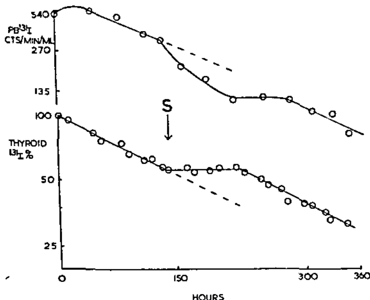


FIG. 6. Injection of stilboestrol (5 mg subcutaneously at ↓) results in an inhibition of the release of thyroidal ^{131}I and a fall in $PB^{131}I$ levels (From data on Brown-Grant, 1955*a*)

levels when release began again. Similar arguments can be adduced, as in the case of the stress experiments, to show that the changes in thyroid activity occur not in accordance with a "feed-back" mechanism but rather that such a mechanism should be acting to oppose the observed changes.

The evidence, then, is that any variations in the rate of peripheral utilization of thyroid hormone that may occur under conditions of stress or the influence of cortisone or

was followed by a resumption of ^{131}I release. Such an experiment provides additional evidence for the delicate control of homeostasis afforded by the "feed-back" system of the thyroid pituitary axis.

(2) *The effect of stress*

Rabbits were subjected to forced immobilization for 48 hours (emotional stress) or laparotomy under ether anaesthesia (physical stress) during the course of an experiment

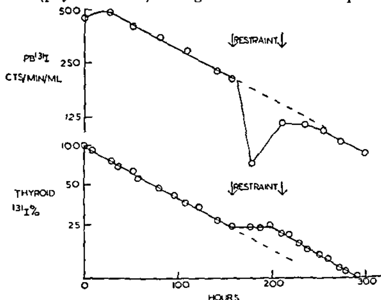


Fig. 5. The effect of "emotional stress" (forced immobilization) on the rate of release of thyroidal ^{131}I in the rabbit. Note the associated fall in PB^{131}I level (From Brown-Grant, 1955*a*.)

(Fig. 5). The expected inhibition of the release of thyroidal radioiodine was observed, and was associated with a fall in the PB^{131}I below the predicted value. When the release of ^{131}I began again, blood levels returned to normal. Thus the decrease in thyroid activity was associated with a reduced level of circulating hormone in the peripheral blood. It does not appear possible that the inhibition produced by these particular stimuli can be related to changes in peripheral metabolism

without the intervention of the hypothalamus. Evidence for this statement has been obtained from work in two laboratories. Von Euler and Holmgren (1956*a*, *b*) working in Stockholm have studied the effect of intrapituitary and intrahypothalamic injections of small amounts of thyroxine in conscious rabbits on the rate of release of thyroidal radioiodine. They showed that, whereas injections into the hypothalamus had no effect, intrapituitary injections caused a marked inhibition of release. The dose of thyroxine needed to elicit this effect produced no change in the rate of release when injected systemically. Rabbits were also studied in which, after hypophysectomy, pituitary tissue from the animals own young had been successfully implanted in the anterior chamber of the eye. In all cases in which viable tissue was found to be present at autopsy, the injection of thyroxine was found to produce a temporary inhibition of ^{131}I release from the thyroid. Simultaneously studies were being carried out in Professor G. W. Harris's department at the Institute of Psychiatry, London, on the thyroid function of rabbits in which the pituitary stalk had been cut and precautions taken to prevent the regrowth of the portal vessels (Harris, 1955*b*; Harris. Reichlin and Brown-Grant, 1955). Among other findings, the most important for the present discussion was that in none of the nineteen animals studied was the inhibitory response to the injection of thyroxine abolished, despite the absence or gross reduction of the vascular connections between the pituitary and the hypothalamus. It appears, then, that the pituitary can respond directly to an excess of thyroxine and independently of the hypothalamus. In neither group of experiments was any diminished sensitivity to thyroxine revealed as compared with intact animals.

A hypothetical model of the hypothalamic—pituitary—thyroid axis

Four separate series of observations have to be considered. First, those reactions of the thyroid which can easily be

stilboestrol are not effective, under the conditions of the experiments described above in causing changes in PBI that could explain the results obtained. Other observations support this view; thus Engstrom and Markardt (1955) and Ingbar and Freinkel (1955) found that cortisone (or ACTH) had no effect on the rate of metabolism of exogenous thyroxine in treated cases of primary or secondary myxoedema, and Engstrom and Markardt (1954) found that oestrogens had no effect on PBI levels in these cases. Lashof, Bondy, Sterling and Man (1954) observed no effect of strenuous exercise on the rate of radiothyroxine metabolism in intact men, and suggest that the decreased metabolism observed after forced swimming of thyroidectomized rats in earlier work (Bondy and Hagewood, 1952) was due to the raised temperature of the water (27° C). Their observation that large doses of cortisone decrease the metabolism of thyroxine in thyroidectomized rats, however, does not appear to have been either confirmed or denied by any further investigations in this species.

The site of action of thyroxine

A simple "feed-back" does not seem to provide a mechanism for the second group of experimentally induced changes discussed above. However, more detailed examination of the site of action of thyroxine may enable these two groups of findings to be reconciled in a single hypothesis. Fig. 1 of this paper presents the essence of a "feed-back" mechanism, applied in this instance to the thyroid-pituitary axis; with appropriate modifications this could also represent, at least in part, the relationship of the gonads or adrenals to the pituitary. It will be noted that the target organ hormone level is represented as acting both upon the hypothalamus and upon the pituitary directly. Until recently there was no evidence to suggest which of these two alternative sites was the true point of action or whether both were involved. It can now be stated definitely that alterations in the blood level of thyroid hormone can react directly upon the anterior pituitary

blood supply to the anterior pituitary is portal vessel blood (Harris, 1955*a*). Anatomically, this arrangement would appear well adapted to act either as suggested here or in the transfer of the postulated neurohumoral transmitter substances. There is evidence that the hormone brought to the region of the primary plexus (median eminence) can be removed from the blood and concentrated there and in the posterior pituitary. Several workers have described a localization of radioactivity in this region after the injection of radiothyroxine (Gross and Pitt-Rivers, 1952; Courrier, 1952), and this has been shown chromatographically to be due to thyroxine. Triiodothyronine is similarly concentrated in the rabbit (Taurog, Harris, Tong and Chaikoff, 1956). That this concentration does not occur in all species does not necessarily exclude the presence of a mechanism for reducing the hormone content of the portal vessel blood, as the abstracted hormone may be more rapidly metabolized or returned to the general circulation in these species.

If the concentration of thyroid hormone in the blood supplying the pituitary is normally reduced by a constant amount during its passage through the primary plexus of the portal vessels, the anterior pituitary will be accustomed to an environment of lower content than the general blood level. Certain of the experimental findings discussed earlier may be re-examined on the basis of this hypothesis. Transplantation of the pituitary or pituitary stalk section, followed by the establishment of a non-portal blood supply to the gland, will result in its exposure to blood of a higher hormone content (Fig. 7). This could be at least a partial explanation of the reduced thyroid activity observed under these circumstances (Schweizer and Long, 1950; Harris and Jacobsohn, 1952; Greer, Scow and Grobstein, 1953; von Euler and Holmgren, 1956*b*, for transplants; Westman and Jacobsohn, 1938; Westman, Jacobsohn and Okkels, 1942; Brolin, 1945; Barrnett and Greep, 1951; Harris, 1955*a*, *b*, for stalk section). Hypothalamic lesions without damage to the portal vessels may also result in thyroid hypofunction (see references

explained on the basis of a "feed-back" mechanism; secondly, the reactions to oestrogens and adrenal steroids, and to stress which cannot apparently be so explained; thirdly, the evidence that firmly establishes the capacity of the isolated pituitary to respond to changes in the blood level of thyroxine. Finally, there is the considerable body of evidence that the hypothalamus is concerned in the regulation of thyroid function, its effects presumably mediated in some way through the portal vessels. The older work suggestive of such control has been presented by Harris (1948, 1955*a*) and the recent studies of Greer (1951, 1952) and Bogdanove and Halmi (1953) in the rat, Ganong, Fredrickson and Hume (1955) (dog) and Fortier, Harris and MacDonald (unpublished) in the rabbit have established beyond reasonable doubt that certain hypothalamic lesions reduce the level of thyroid activity and diminish the response to various stimuli such as thiouracil treatment The final step, the demonstration of increased thyroid activity following electrical stimulation of the hypothalamus, has been recently achieved (see Harris and Woods, 1956).

The following speculative attempt to combine these findings is based on a suggestion made to the author and Professor Harris by Dr. Curt von Euler in 1952. It must be emphasized, however, that the responsibility for the present digression into the realms of speculation is solely the author's. The essence of this hypothesis is that the rôle of the hypothalamus in the control of pituitary TSH secretion is to remove thyroid hormone from the arterial blood supplying the primary plexus of the hypophysial portal system and so expose the pituitary to blood of a modified thyroid hormone content. Certain details and consequences of this hypothesis will be considered. First, and primarily, it is clear that the anterior pituitary secretion of TSH can alter in response to changes in the thyroid hormone content of the blood to which it is exposed. Secondly, the vascular anatomy of the region appears admirably adapted to permit such a functional relationship; in several forms (e.g. man and rat) virtually the whole of the

compatible with this view. Cortisone and physical stress do still appear to be effective after stalk section or transplantation of the pituitary (Brown-Grant, Harris and Reichlin, 1955; von Euler and Holmgren, 1956*b*) but it is possible that

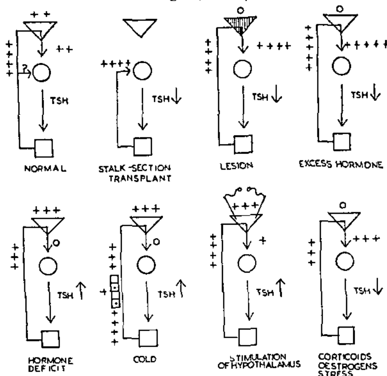


FIG. 7. To illustrate the suggested mode of action of various experimental procedures in terms of the hypothetical "feed-back" mechanism discussed in the text.
▽—Hypothalamus and portal vessels
○—Anterior pituitary
□—Thyroid

cortisone may exert a direct effect on the thyroid which is not connected with either a reduction in pituitary TSH secretion or the response of the thyroid to either exogenous or endogenous TSH, while laparotomy may alter the sensitivity of the gland to TSH (Brown-Grant, Harris and Reichlin, 1955).

previously cited), and the mechanism here may be the destruction of those parts of the hypothalamus responsible, possibly by some metabolic process, for the reduction of portal vessel hormone levels. Electrical stimulation could act by enhancing this process, lowering the level abnormally and hence stimulating the pituitary to secrete extra TSH (Fig. 7).

The reaction of the thyroid to variations in the systemic level of hormone can occur without this mechanism. In the intact animal, however, it appears that it may play a rôle synergistic to the general change. Thus the response to goitrogen administration is diminished in rats with hypothalamic lesions (Greer, 1952; Bogdanove and Halmi, 1953) or after stalk section (Barrnett and Greep, 1951). The response to cold, as was suggested earlier, may occur as a result of a fall in the systemic level of hormone. Increased activity of the hypothalamus could be of importance in additionally reducing the level in the blood to which the pituitary is exposed. This might be a more rapid process than the fall in peripheral levels. Abolition of part of this dual mechanism could result in a less sensitive response to cold exposure and explain the failure of Uotila (1940) and Brolin (1945) to detect signs of hyperactivity in stalk sectioned rats in the cold and the reduced response observed by Barrnett and Greep (1951). Von Euler and Holmgren (1956*b*) found no increase in thyroid activity when rabbits with ocular transplants of the pituitary were exposed in acute experiments; longer exposure might have elicited an increase via the possibly more sluggish mechanism of a fall in systemic PBI levels.

Finally, the changes in thyroid activity produced by cortisone and oestrogens in large doses or by stress could be the result of these influences causing a reduction in the extent to which the hypothalamus lowers the portal vessel blood level of hormone, with subsequent depression of pituitary TSH secretion in response to a raised level of hormone despite the presence of a lowered systemic level. Oestrogens and emotional stress no longer reduce thyroid activity after pituitary stalk section in the rabbit (Harris, 1955*a*, *b*), a finding which is

BROLIN, S. E. (1945). *Acta Anat.*, Suppl 3, 165.
BROWN-GRANT, K. (1955a). Thesis for degree of M D, University of Cambridge.
BROWN-GRANT, K. (1955b). *J. Physiol*, **127**, 352.
BROWN-GRANT, K. (1956). *J. Physiol.*, **131**, 52.
BROWN-GRANT, K., EULER, C. VON, HARRIS, G. W, and REICHLIN, S. (1954). *J. Physiol.*, **126**, 1.
BROWN-GRANT, K., and GIBSON, J G (1955). *J. Physiol.*, **127**, 328.
BROWN-GRANT, K., HARRIS, G. W, and REICHLIN, S (1954a). *J. Physiol.*, **126**, 29.
BROWN-GRANT, K., HARRIS, G W, and REICHLIN, S. (1954b). *J. Physiol.*, **126**, 41.
BROWN-GRANT, K., HARRIS, G. W, and REICHLIN, S. (1955). *In* Harris, G. W. (1955b)
[illegible]
[illegible]
docrinology, **57**, 355
GIBSON, J. G., HARRIS, G. W, and SKYNNER, A C R (1954) Unpublished observations quoted by Harris (1955a).
GOLDBERG, R. C., GREEP, R O., and NAY, L B (1953). *Proc Soc exp. Biol., N.Y.*, **84**, 621.
GOTTSCHALK, C. W., and RIGGS, D S. (1952) *J. clin. Endocrin*, **12**, 235.
GREER, M A. (1951). *Proc. Soc exp Biol., N Y.*, **77**, 603.
GREER, M. A. (1952). *J clin. Endocrin*, **12**, 1259.
GREER, M A, SCOW, R O., and GROBSTEIN, C. (1953). *Proc. Soc. exp. Biol, N.Y.*, **82**, 28.
GROSS, J, and PITT-RIVERS, ROSALIND. (1952). *Brit. med. Bull.*, **8**, 136.
GYLLENSTEN, L. J. (1953). *Acta Anat*, **17**, Suppl. 18.
HALMI, N. S., and SPIRTOS, B. N. (1954) *Endocrinology*, **55**, 613.
HARPER, E. O, and MATTIS, P A. (1951). *Fed Proc.*, **10**, 306.
HARPER, E O, MATTIS, P. A, and BOEHNE, J. W. (1952) *Fed. Proc.*, **11**, 353.
HARRIS, G W. (1948) *Physiol Rev*, **20**, 139.
[illegible]

This highly speculative attempt to provide a working hypothesis of the functioning of the hypothalamic-pituitary-thyroid axis (Fig. 7) has been presented only in the hope that its shortcomings may be quickly exposed and a more valid and better-founded one replace it. In its defence can be offered only its superficial consistency and the fact that at least two aspects are readily open to experimental investigation. The technically difficult, but not impossible, problem of the collection and assay of portal vessel blood for its content of stable or radioactive thyroid hormone could disprove the essential basis of the theory very quickly. The second has *already been investigated on a limited scale; that is the* determination of the uptake of thyroid hormone by the median eminence and posterior pituitary under different physiological conditions. To date, such information as is available tends rather to support the thesis developed in this paper, in that, although the uptake of radiothyroxine by the brain in general is decreased in thyroidectomized rabbits, the uptake by the tuber cinereum is unaffected and that of the posterior pituitary is increased (Harper and Mattis, 1951; Taurog, Harris, Tong and Chaikoff, 1956). In contrast the uptake in hyperthyroid animals is reduced (Harper, Mattis and Boehne, 1952).

Acknowledgements

[illegible]

REFERENCES

Aron, M., Caulert, C. van, and Stahl, S. (1931). *C.R. Soc. Biol., Paris,* 107, 64.

[illegible] *J. Physiol.*, 167, 569.

[illegible] *ocrinology*, 53, 274.

[illegible] *Proc. Soc. exp Biol*, [illegible]

BROLIN, S. E. (1945). *Acta Anat*, Suppl 3, 165.
BROWN-GRANT, K. (1955*a*). Thesis for degree of M.D., University of Cambridge.
BROWN-GRANT, K. (1955*b*). *J. Physiol.*, 127, 352.
BROWN-GRANT, K. (1956). *J. Physiol*, 131, 52.
BROWN-GRANT, K., EULER, C. VON, HARRIS, G. W., and REICHLIN, S. (1954). *J. Physiol*, 126, 1.
BROWN-GRANT, K., and GIBSON, J G (1955). *J Physiol.*, 127, 328.
BROWN-GRANT, K., HARRIS, G W., and REICHLIN, S. (1954*a*). *J. Physiol.*, 126, 29.
BROWN-GRANT, K, HARRIS, G W., and REICHLIN, S. (1954*b*). *J. Physiol.*, 126, 41.
BROWN-GRANT, K., HARRIS, G. W, and REICHLIN, S. (1955). *In* Harris, G. W. (1955*b*)

4,

D'ANGELO, S. A. (1955) Brookhaven Symposia in Biology, 7, 9.

docrinology, 57, 355.
GIBSON, J. G., HARRIS, G. W., and SKYNNER, A. C. R. (1954). Unpublished observations quoted by Harris (1955*a*).
GOLDBERG, R. C., GREEP, R O, and NAY, L B (1953). *Proc. Soc. exp. Biol., N Y*, 84, 621.
GOTTSCHALK, C W, and RIGGS, D. S (1952). *J. clin Endocrin*, 12, 235.
GREER, M. A. (1951). *Proc Soc. exp. Biol, N Y*, 77, 603
GREER, M. A (1952) *J. clin. Endocrin*, 12, 1259
GREER, M. A., SCOW, R. O, and GROBSTEIN, C (1953). *Proc. Soc. exp. Biol, N Y*, 82, 28.
GROSS, J, and PITT-RIVERS, ROSALIND. (1952) *Brit. med Bull.*, 8, 136.

355
HARRIS, G. W. (1948) *Physiol Rev.*, 20, 139

Harris, G. W., and Jacobsohn, Dora. (1952) *Proc. Roy. Soc. B*, **139**, 263.

Harris, G. W., Reichlin, S., and Brown-Grant, K. (1955). *J. Lab. clin. Med.*, **46**, 822.

Harris, G. W., and Woods, J. W. (1956). This Colloquium, p. 3

Hoskins, R. G. (1949). *J. clin. Endocrin.*, **9**, 1429

Ingbar S H., and Freinkel, N. (1955) *J clin. Invest.*, **34**, 1375

[illegible]

36, 617.

Nièpce, C. (1851) London: Ballière. Quoted by D'Angelo (1955).

Rand, C. G., Riggs, D. S., and Talbot, N B. (1952). *Endocrinology*, **51**, 562.

Reichlin, S (1954) Thesis for degree of Ph.D., London University.

Reichlin, S., and Reid, A. A (1955). *Proc. Soc. exp. Biol., N.Y.*, **89**, 212.

Sayers, G. (1950). *Physiol. Rev.*, **30**, 241.

Schweizer, M., and Long, M. E. (1950). *Endocrinology*, **47**, 454.

Stevens, C. E., D'Angelo, S A , Paschkis, K E , Cantarow, A., and Sunderman, F. W. (1955). *Endocrinology*, **56**, 143

Taurog, A., Harris, G. W, Tong, W., and Chaikoff, I. L. (1956). [illegible]

Uoti [illegible]

Van [illegible] logy, **7**, 30

Van [illegible] *acol. Rev*, **7**, 301.

Westman, A., and Jacobsohn, Dora (1938). *Acta path. microbiol. scand.*, **15**, 435.

Westman, A., Jacobsohn, Dora, and Orkels, H. (1942). *Acta path. microbiol. scand.*, **19**, 42.

Wolf, O., and Greep, R. (1937). *Proc. Soc exp. Biol., N.Y.*, **36**, 856.

DISCUSSION

[illegible] caused by oestrogen ob- [illegible]

we quite agree that the anterior pituitary is not the only site of regulation We do not share von Euler's conclusion that the direct action of thyroxine on the anterior pituitary is the only way whereby thyroxine regulates thyroid secretion.

B.-Grant. I do not think Prof von Euler contends that the action of thyroxine on the anterior pituitary is the only regulating mechanism. He observed, for instance, that in his transplanted animals he abolished the effects of cold or emotional stress on thyroid activity.

B.-Grant. You certainly do see a change in the slope if you block the

rabbit. Using three different methods, we found that about 10 per cent of the radioactive iodine secreted in the form of hormone was degraded and reaccumulated by the thyroid in the form of ^{131}I. That means that a

release, whereas, in point of fact, cortisone administration causes a decrease in the rate of release. So the effects, in that instance anyway, of changes in reaccumulation do not help you at all to explain your experimental findings.

Gross: I think Dr. Brown-Grant's suggestion of the hypothalamic region as a thyroid hormone clearer is a very interesting one. In this connection, we have some time studies on the concentration of triiodothyronine in the rabbit, the guinea pig, the rat, and a few scattered observations in the monkey. What we find is that in all these species, with triiodothyronine, one can demonstrate concentrations in the anterior and posterior pituitary, and possibly also in the hypothalamus. With the rabbit it is rather nice; the time curve for the posterior pituitary rises above and stays above that of the anterior pituitary. Thus would

B.-Grant: Have you any suggestions as to the possible result of experiments on blood collected from the cut stalk to see whether it has a different content of thyroid hormone from the arterial blood? Portal vessel blood has been collected in the dog for other reasons, and it was attempted in Prof. Harris's department on the rabbit It certainly can be done although it is difficult.

Gross: A logical experiment, which I think you suggested, would be to do stalk sections and see whether in the case of triiodothyronine there is a depression of entry of radioactivity into the anterior pituitary.

B.-Grant: Your finding of an increased content in the anterior

thyroid hormone is thyroxine.

Gross: Quite right.

Harris: When you say triiodothyronine is concentrated in the anterior lobe, do you mean above plasma level?

Gross: Several-fold above plasma. These are unequivocal concentrations.

might be to modify or to poison the cells so that this region of the hypothalamus no longer takes up thyroxine, in which case, somewhat analogous to the state under conditions of stress or after cortisone treatment, a reduced peripheral level might no longer be reflected in a reduced level in the blood to which pituitary is exposed

Maclagan: As I understand it this new theory was really designed to explain the fact that you could have an inhibition of iodine release associated with low levels of peripheral thyroid hormone. Could one not explain that equally well—I am not suggesting that it is a better explanation but an alternative one—by saying that the sensitivity of the pituitary to thyroid hormones is altered by the simultaneous presence of, say, cortisone. That would appear to me to have much the same consequence.

B.-Grant: Yes, certainly that is one way in which the effect of cortisone or stress could be explained, and it is an equally valid explanation in light of present knowledge I do not know whether it explains the effects

there not to explain your results?

B.-Grant I do not think so That is really one of the possibilities that has been raised every so often as a major factor in controlling the thyroid activity—the rate of extra-thyroidal peripheral destruction of TSH—and it is not one that, as far as I can see, has yet been clearly established as of any importance in the normal intact animal.

Querido But the extra-thyroidal clearance is very fast.

B.-Grant: Certainly. I believe Sonnenberg (discussion of paper by D'Angelo (1955) at the Brookhaven Symposium) estimated it might be that something like 98 per cent of TSH did not go to the thyroid but was destroyed elsewhere

Querido: We have had experience of that in normal and thyroid-

established that changes in the extra-thyroidal metabolism of TSH really affect the level of thyroid activity? Do you think that such changes are an important factor in determining the level of thyroid function in normal intact animals?

Querido: I do not think that any factors in thyroid disease are known The kidney is an important factor for the disappearance of TSH from the blood. If you bind the renal artery of normal animals the level stays up much longer although it is not normally excreted in the urine; it is just fixed or handled in some way by the kidney.

B.-Grant: To make that a physiological mechanism, though, you would have to postulate a gross renal arterial spasm to mimic your experimental conditions

Querido: Yes.

GENERAL DISCUSSION

Greer: The question of TSH assays in the blood was brought up

subjects the response starts at 1 U.S P. unit of TSH/day, given intramuscularly. This presumably means that the endogenous secretion is below that level. Even assuming that endogenous TSH secretion is equivalent to 1 unit/day and that this is immediately distributed in 15 litres of extracellular fluid, there would only be 0·06 milliunits/ml. This is below what might be expected to be picked up by current methods of bioassay.

continuously over a 24-hour period. It is also probable that the distribution would involve more than just the extracellular space. The actual level one would expect in the blood would thus be very much less than 0·06 milliunits/ml

That leads me to the question of whether we can actually put too much faith in current bioassays of blood TSH. The figures are quite discrepant between what one seems to pick up and what one might calculate should be there. I wonder if those who are doing blood bioassays might comment on that.

Querido· Of course, the question always comes up whether what one determines in serum is really TSH. The argument we had for it is as follows: we concentrated it from serum on the basis of protein fractionation on properties known to us from hypophyseal TSH preparations.

Secondly, in several cases of acromegaly which were active acromegalics but were not thyrotoxic (as sometimes happens in acromegalics), there was no increased response in the animal on which the assay of the serum was done From this we conclude that the factor causing acromegaly is not a factor in the TSH assay.

We have also tried with ACTH and gonadotrophins with negative results. However, there still might be existent in the serum an unknown factor that gives thyroid stimulation and imitates a TSH response.

Albert: Dr. Purves, what is the sensitivity of your thyrotrophin

assay? You said this morning that you got thyrotrophin effects from serum from patients with thyrotoxicosis.

Purves: Not consistently. Dr. Adams now states that he has obtained responses with 2 μg. of the U.S P. thyrotrophin reference; that is 2 μg. of material of which 20 mg. is one unit, so that is 10^{-4} of a unit We could, therefore, detect 10^{-4} unit per ml. of serum.

Querido This is the famous difficulty in TSH questions. The assay as we did it comes down to 2–8 μg /ml. provisional U.S.P. standard. And it was of the same order as Gilliland and Fraser claimed with their chicken assay It was somewhat above the figures Dr. Purves had with the cytological method

Purves The cytological assay of the guinea pig thyroid does not detect any TSH in normal human beings or in thyrotoxics except that one, rarely, gets a response of unknown significance. The assay method which we are now using is that of Adams and Purves (1955. *Endocrinology*, 57, 17–24), and it is this method which has the sensitivity I have just mentioned. With this method one can assay the blood of rats and get responses which make sense, they go up when they should, and go down when the hormone should not be there. Levels in the human are definitely lower but it does not seem that they should be impossible to detect.

Greer. If one gets a level of 2–3 milliunits/ml., that would still be roughly at least 50 times as much as one would expect from this theoretical one unit calculation Either my calculations or the bioassays would seem to be in error.

Querido It is still open to discussion, of course, whether one is assaying TSH or not The only point that can be made is that this material gives thyroid stimulation in the assay animal. Further, that by assay on two qualities of thyroid stimulation, either by uptake or by discharge, the same order of magnitude is found; that is the only thing one can say.

The only thing I can add to that is that I do not have the impression that it is growth hormone, but that is only on the basis of assay of serum in active acromegaly. But still an X factor could be possible.

First we studied a purified anterior lobe TSH preparation. The TSH activity moved on electrophoresis with about the same speed as β-globulin under identical circumstances. We are aware that this does not necessarily mean the same for serum TSH

Then we had a patient with a postoperative myxoedema with a tremendous quantity of TSH-like material in the serum—an amount which could easily be detected by the injection of unconcentrated serum. This serum was then analysed in paper electrophoresis. The activity was so high that we could just elute the

paper in different fractions and do an assay on each fraction. We found the activity on a spot near to the β-globulins. From those two points we decided to concentrate serum with Cohn's method. The assay is done in the mixture of II + III, IV 1 and IV 4

The next point was that a curve of this strongly active plasma showed the same regression as our standard TSH material in the assay. Also, we have never been able to show any activity in the material in other fractions.

So it is from all these indirect arguments that we conclude that

and Lameyer, L. D F. (1956) *Proc R Soc. Med.*, 49, 209).

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Iodine prophylaxis was exercised in certain areas in Holland through iodinization of drinking water in the years preceding World War II. Difficulties in supply of potassium iodide caused an interval of insufficient prophylaxis, which was re-established after the war by using iodized baker's salt. The endemic goitre problem was re-studied in the past five years. During that period Miss Terpstra had the opportunity to do

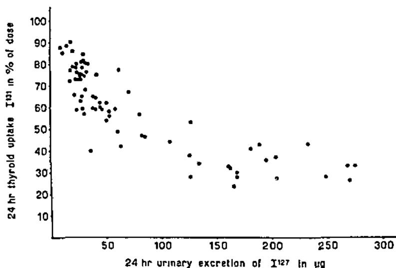


FIG. 1. Relation of ^{127}I excretion per 24 hours and 24 hours ^{131}I uptake in neck.

observations in groups of young people, comparing iodine-deficient areas with regions adequately supplied The 24-hour urinary excretion of ^{127}I was used as criterion of iodine deficiency. The thyroid gland function was studied by means of ^{131}I uptake and urinary excretion, as well as by the determination of $PB^{127}I$ and the appearance of $PB^{131}I$ in the serum. The same type of inverse relationship between average (2–5 collections of 24-hour urine) ^{127}I excretion and 24-hour ^{131}I uptake in the gland was found as Stanbury *et al.* (1954) reported from Mendoza (Fig. 1). Some detailed observations indicated that probably increased ^{131}I avidity during iodine

HORMONE SYNTHESIS IN THE IODINE-DEFICIENT THYROID GLAND

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The interesting study by Stanbury, Brownell, Riggs and their Argentinian co-workers (1954) of the dynamics of iodine metabolism in an endemic goitre area led to the conclusion that adaptation to iodine deficiency seems to be entirely dependent upon the thyroid. The kidney, according to their observations, plays no rôle in adaptation to iodine deficiency. Even in severe iodine deficiency the thyroid gland seems to be able to maintain a euthyroid condition that seems to be achieved through a hormone secretion equivalent to a normal amount of thyroid hormone activity. In their studies there was no indication that this was necessarily achieved through a change in the composition of the circulating hormone. The PBI values obtained showed a normal mean value. Stanbury *et al.* (1954) state, however, that the standard deviation was larger than in non-goitrous normals, and that low PBI values tended to occur in patients with high uptakes.

Adaptation of the thyroid is achieved by increased avidity of the gland for iodide, which results in a greater share for the gland of both dietary iodide and iodide from thyroid hormone degradation. Obviously there will be a level of dietary iodide so low that this mechanism will not be able to provide the thyroid with enough iodide for an adequate hormone production. In the compensated iodine-deficient gland increased uptake leads to a normal iodine supply for hormone synthesis, and there seems to be no reason to assume changes in intra-thyroidal metabolism. In the decompensated gland, however, intra-thyroidal metabolism puts forward many questions.

Table I

EFFECT OF IODINE DEFICIENCY ON $PB^{127}I$ AND URINARY IODINE EXCRETION IN HUMANS

	Goitre group (34 subjects)	*Control group (11 subjects)*
$PB^{127}I$ μg./100 ml	5·6 ± 0·7	5·7 ± 1·1
Urinary excretion of ^{127}I in μg per 24 hr.	30 ± 9·4	176 ± 42·4

with high iodine excretion (Table I). In our 24-hour and 48-hour serum $PB^{131}I$ data in iodine-deficient individuals we were unable to discover any differences from such data obtained in normals (i.e. below 0·35 per cent dose/l.). This might indicate that total thyroid iodine content in the investigated iodine-deficient cases was about the same as in normal individuals (Table II).

Table II

DATA ON ENDEMIC GOITRE IN HOLLAND

Sex	*Age*	*Thyroid size (times normal)*	*Total urine ^{127}I excretion per 24 hr* * μg	*$PB^{127}I$* μg	*$PB^{131}I$ after 24 hr.* % *dose/l*	*Conversion ratio after 24 hr.*† %
♀	23	2–3	33	6·0	0·06	64
♀	31	3	24	6·0	0·09	79
♀	21	5	27	5·1	0·026	82
♀	16	4–5	32	5·3	0·045	42
♀	21	4	26	6·4	—	—
♀	26	2	14	5·6	0·11	87
♀	25	3	17	6·8	0·044	81
♂	37	3–4	17	5·8	0·04	65
♀	16	6	15	5·3	0·047	50
♀	32	4–5	42	4·9	0·078	59

* Average figures of 2 or 3 observations

† Conversion ratio = $\frac{\text{serum } PB^{131}I}{\text{total serum } ^{131}I}$

deficiency may precede enlargement of the thyroid gland (Fig. 2). Both patients had low ^{127}I excretion and thyroidal

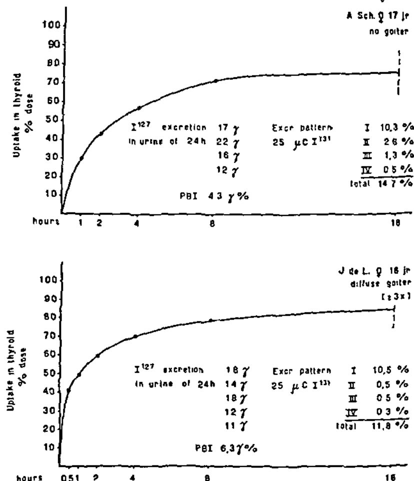


FIG. 2 Increased thyroid iodine avidity, without and with goitre, in iodine-deficient humans.

^{131}I uptakes were more than 70 per cent. The patient above, however, had no palpable enlargement of the thyroid gland.

The PB^{127}I in thirty-four subjects with goitre and low iodine excretion was not different from a small normal series

Table III

EFFECT OF IODINE DEFICIENCY ON ^{131}I PATTERN

Diet for 6 months	Hours after injection	Thyroid						
		Total wt. in mg.	^{131}I % dose in one lobe	% ^{131}I in chromatographic fractions				
				Iodine	Monoiodo-tyrosine	Diiodo-tyrosine	T_3	T_4
Remington	9	59·1 ± 5·2	17·7 ± 6·0	13·7 ± 1·7	31·7 ± 3·5	33·3 ± 3·5	2·8 ± 0·7	2·5 ± 0·7
	24	41·4 ± 4·0	22·6 ± 4·4	18·7 ± 1·4	33·5 ± 2·6	28·6 ± 2·7	3·5 ± 0·5	2·9 ± 0·5
	96	35·2 ± 9·4	12·8 ± 5·4	14·7 ± 2·4	36·4 ± 3·4	24·0 ± 1·4	4·3 ± 0·5	4·0 ± 1·1
Remington + 1 mg. I per kg. food	9	15·7 ± 4·6	5·8 ± 2·0	9·1 ± 1·1	32·1 ± 2·8	46·3 ± 3·6	1·9 ± 0·4	4·2 ± 1·4
	24	16·5 ± 1·9	7·9 ± 1·1	10·0 ± 2·2	24·9 ± 3·4	47·4 ± 2·8	2·2 ± 0·5	4·6 ± 1·4
	96	18·8 ± 3·2	5·8 ± 1·2	11·9 ± 1·3	23·0 ± 1·7	46·1 ± 1·0	2·7 ± 0·5	5·5 ± 1·7

All figures indicate mean ± S.D. of 4 or 5 animals.

The problem as to whether *severe* iodine deficiency changes the biosynthesis of thyroid hormones was studied in rats by Dr. Schut. Young female rats of 150 g. were given Remington diet 342, which contained 0–40 μg. I per kg., for 6–12 months. Control animals received the same diet, but with addition of 1 mg. iodine as KI per kg. food. Thyroids were analysed after intravenous injections of carrier-free ^{131}I. The glands were digested with pancreatic powder at pH 8·4, extracted with butanol saturated with sodium thiosulphate, and chromatographed in butanol-acetic acid and/or in the butanol-dioxan-ammonia system on Whatman No. 1 or No. 3 paper. Counting of radioactivity was done in a well-type scintillation counter. Activity was measured per cm. paper or of spots obtained with carrier stained by sulphanil diazonium chloride and palladium chloride. The chromatograms were, in some experiments, analysed for ^{127}I content with the Barker acid digestion method. The paper contained only 0·13 μg. iodine per 15 cm.[2]

The results obtained are given in Table III.

In the iodine-deficient animals the activity of the monoiodotyrosine fraction in the gland is equal to or higher than that found in the diiodotyrosine fraction. In the animals receiving extra KI (and having normal thyroid weights) the diiodotyrosine fraction of the gland is always more active than the monoiodotyrosine fraction. The same change is observed in the relationship between the triiodothyronine (T_3) and thyroxine (T_4) fraction.

Analysis of the serum shows, in the iodine-deficient animals, that already at 9 hours a constant ratio of triiodothyronine and thyroxine (approximately 1 : 10) is established (Table IV) In the non-deficient animals the ratio of activity is different between 24 and 96 hours, and we do not know at what point the maximum value is achieved. The deficient glands, furthermore, at 96 hours still maintain high total ^{131}I and PB^{131}I values in the serum. This, together with the low PB^{127}I in the serum and the constant ratio of triiodothyronine and thyroxine in the serum, seems to indicate the continuous thyroid

an increase in size only after lowering of serum PBI values. In this respect the clinical data are different.

While Schut's experiments were in progress, Leloup and Lachiver (1955) published similar data for monoiodotyrosine and diiodotyrosine activities in the thyroids of iodine-deficient and control animals. They conclude that more tri-iodothyronine is formed. Our data support the view that indeed more monoiodotyrosine than diiodotyrosine is present in the gland, as indicated by analyses of the stable compounds. The serum activity data on triiodothyronine and thyroxine, however (change of the ratio triiodothyronine : thyroxine in

Table V

EFFECT OF LONGSTANDING IODINE DEFICIENCY ON THYROID METABOLISM IN RATS

Diet for 12 months	Serum	Thyroid					
	$PB^{131}I$	wt in mg		^{131}I content % dose		^{127}I content in µg	
		single rats	combined	Monoiodo-tyrosine	Diiodo-tyrosine	Monoiodo-tyrosine	Diiodo-tyrosine
Remington	1·1 1·6	74·8 52·1	126·9	13·4	8·6	1·70	0·73
	0·9 0·9	72·0 73·5	145·5	15·3	12·6	0·83	2·13
	0·8 1·0	69·5 53·9	123·4	14·8	11·9	0·85	0·77
Remington + 1 mg iodine per kg. food	3·1 3·5	16·7 27·6	44·3	3·2	4·7	16·8	26·6
	1·8 2·9	22·4 17·2	39·6	3·1	3·3	10·0	20·2
	3·5 3·5	25·8 25·1	50·9	1·3	1·9	8·9	13·8

Table IV

EFFECT OF IODINE DEFICIENCY ON SERUM ^{131}I PATTERN

Diet for 6 months	*Hours after injection*	*Serum*			
		^{131}I % dose in 1 ml.	*% ^{131}I in chromatographic fractions*		
			I	*T_3*	*T_4*
Remington	9	0 24 ± 0·06	10 ± 9	7 ± 1	72 ± 6
	24	0 30 ± 0 02	5 ± 1	7 ± 0	82 ± 2
	96	0 15 ± 0·02	6 ± 1	8 ± 2	80 ± 2
Remington + 1 mg. I per kg. food	9	0 23 ± 0 05	95 ± 2	1 ± 0	1 ± 1
	24	0 039 ± 0 020	69 ± 12	4 ± 3	17 ± 9
	96	0 021 ± 0 005	25 ± 9	10 ± 4	53 ± 8

All figures indicate mean ± S.D. of 4 or 5 animals

release of compounds with high specific activity, probably through decreased iodine store.

Table V gives Dr. Schut's data in another series, where ^{127}I was estimated in animals treated in the same way. The blood PB^{127}I in the deficient animals was distinctly lower than in the control group. The glands of two animals were pooled for analyses. Again, more activity was found in the mono-iodotyrosine fraction than in the diiodotyrosine fraction, and in four of the six animals monoiodotyrosine was indeed more than diiodotyrosine. In the control animals the data on activity and monoiodotyrosine and diiodotyrosine are the other way round.

If we try to correlate the clinical studies with available experimental data, we have to refer to Money, Rall and Rawson (1952), Halmi (1954), and VanderLaan and Caplan (1954). These authors all observed increased avidity for iodide prior to growth of the thyroid when the animals were on an iodine-deficient diet. Money, Rall and Rawson, however, observed

I am sorry to interrupt but I do not think that is the usual distribution in the normal thyroid for the percentage of thyroxine present in

figures.

Gross: In some of our work on the autoradiographs we used Remington plus 20 μg./day/animal, and 20 μg./day slowed down the thyroid very considerably

Taurog: You mean that even at 96 hours after ^{131}I you would find such a low percentage—5 per cent of the gland's iodine present as thyroxine.

Gross. I have no data on fractionation, only on the formation of thyroglobulin. In the autoradiographic studies we did, giving 20 μg. of iodine with the Remington diet, the iodination of thyroglobulin was slowed down considerably Whether it would reflect in these figures or not, I do not know.

Querido: Yes.

Taurog. With regard to that, did you take the area of the paper and do the ^{131}I and ^{127}I on the same piece of paper?

Querido: Yes, that is right.

Taurog: What order of magnitude of iodine were you determining on the paper?

Taurog You mean you made an extract of the gland with butanol and you delivered that on the paper?

Querido: Yes.

Taurog. And you had the equivalent of what part of the gland on the paper?

Querido The full amounts of two glands.

Taurog: Well, that is quite a trick!

Querido We get in the order of nearly 1 μg., and the background of

favour of thyroxine) are very difficult to interpret, and we are not prepared to say whether or not they indicate lowered triiodothyronine secretion by the thyroid gland.

REFERENCES

HALMI, N. S. (1954). *Endocrinology*, 54, 216.

LELOUP, H., and LACHIVER, F. (1955) *C.R. Acad Sci., Paris*, 241, 509

MONEY, W. L., RALL, J. E., and RAWSON, R. W. (1952). *J. clin. Endocrin.*, 12, 1495.

STANBURY, J. B., BROWNELL, G. L., RIGGS, D. S., PERINETTI, H., ITOIZ, J., and DEL CASTILLO, E. B. (1954). Endemic Goiter. Cambridge, Mass · Harvard University Press.

VANDERLAAN, W. P., and CAPLAN, R. (1954) *Endocrinology*, 54, 437.

DISCUSSION

Barker: I wonder if the fact that the serum triiodothyronine radioactivity *in your experimental animals is so much lower than the thyrox-*

... a good deal ... than the diiodotyrosine

96 hours.

Querido: Yes. Isn't this the point that you brought up in the analyti- ... many years ago—in 1946—where you had very high iodide

iodine intake.

ENZYMIC ASPECTS OF THYRONINE METABOLISM AND ITS IODINATED DERIVATIVES

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THE metabolism of thyronine and its iodinated derivatives, particularly those which present a hormonal activity, may in theory take place in several ways:

1. modifications of the alanyl side-chain,
2. partial or total deiodination,
3. conjugation with compounds able to react with the phenolic group,
4. modifications of the diphenyl ether structure giving either substitution in the benzene rings by other atoms than iodine, or the splitting of the ether linkage, and perhaps both.

Mechanisms implied in the first suggestion have been explored during the last few years. It has been possible to show the presence in bile of triiodo- and tetraiodopyruvic acids after the injection of ^{131}I-labelled 3 . 5 : 3′-triiodothyronine or thyroxine to rats (Roche *et al.*, 1954*a*) The same compounds were found in urine (Roche and Michel, 1955). The acetic analogue can be produced by incubation of the hormones with slices or rat kidney homogenates (Lardy, 1956) as well as triiodothyroacetic acid by muscular metabolism of 3 : 5 : 3′-triiodothyronine (Roche, Michel and Jouan, 1956) The second pathway was chiefly investigated with regard to the fact that 3 : 5 : 3′-triiodothyronine, which is 5–10 times as active as thyroxine according to the test used, would arise from the peripheral deiodination of the latter. This reaction was studied *in vivo* (Gross and Leblond, 1951; Gross and Pitt-Rivers, 1952; Flock and Bollman, 1955; Kalant, Lee and

the paper is something like 0·1–0 2 μg /15 sq. cm. These figures are approximate, therefore, but they give an order of magnitude Indeed there is more stable monoiodotyrosine than diiodotyrosine present in this digest, and we are not dealing with a faster formation which is not presenting what actually is present.

Lissitzky How did you do the hydrolysis of the thyroid tissue in those experiments?

Querido: Pancreatic powder, pH 8·4.

Lissitzky. I think the discrepancies between the results in the amounts of iodothyronines can be explained by the hydrolysis of thyroid tissue. We observed, with Professor Roche and Michel, that pancreatic hydrolysis is very suitable for the liberation of mono- and diiodotyrosine, but it is very difficult to liberate iodothyronines with this method of hydrolysis. We have done many experiments in order to explore the possibility of liberating all the iodothyronines in free state with pancreatic digestion, but we have not really been able to show that the free amino acids were liberated in such experiments. I should say that perhaps the differences between your results and those from Dr. Taurog's laboratory and other laboratories could be explained by this technical difficulty. [illegible]

[illegible]

extraction. We put the entire material on paper so that we can see what is left at the origin as an indication of what has been left unhydrolysed.

Now, when you did the butanol extraction, did you count the residue? If you count the residue that is left after the butanol you would get an [illegible]

[illegible]

iodothyronines is higher, but alkaline hydrolysis always gives wrong results.

Querido: But that we did not do!

[illegible]

been [illegible]

different in relative amounts seems to be hard to understand

metric and chromatographic purity were strictly checked. Mono- and diiodotyrosine were prepared by iodination of tyrosine and chromatographic separation on cellulose column; mono- and dichlorotyrosine according to Bouchilloux (1955); mono- and dibromotyrosine according to Yagi, Michel and Roche (1953).

Thyroxine, 3 : 5-diiodo- and 3 : 5 : 3′-triiodothyronine were supplied by Messrs. Hofmann la Roche and used after rechromatography.

3′-Mono- and 3′ : 5′-diiodothyronine were synthesized according to Roche, Michel and Wolf (1954) and purified by paper chromatography. 3-Monoiodo- and 3 : 3′-diiodothyronine were kindly supplied by the latter, tri- and tetraiodothyroacetic acid (TRIAC and TETRAC) were kindly given by Mrs. R. Pitt-Rivers.

The ^{131}I-labelled substrates were obtained by iodination of a direct precursor: 3 5-diiodothyronine for thyroxine and 3 . 5 : 3′-triiodothyronine, thyronine for 3′-mono- and 3′ : 5′-diiodothyronine; 3-monoiodothyronine for 3 : 3′-di- and 3 . 3′ : 5′-triiodothyronine; and tyrosine for mono- and diiodotyrosine. The iodination mixture ($^{127}I_2$ + $^{131}I_2$) arose from the exchange between a chloroform solution of $^{127}I_2$ and an aqueous solution of tracer Na^{131}I brought to pH 5–6 (Lissitzky and Roques, 1956). The separation of radioactive iodinated amino acids was done by paper chromatography in an appropriate solvent, followed by elution and concentration of the eluate under reduced pressure.

The composition of incubation mediums will be given in the text or in the legend of the figures. Experiments were always done at 38°, with continuous shaking under aerobic conditions. Experimental details concerning quantitative analysis of amino acids and their characterization have been recently published (Lissitzky, Bouchilloux and Kertesz, 1956).

The quantitative determination of the radioactivity of chromatograms was carried out either by radiochromatography or by densitometry of the films obtained by radioautography.

Sellars, 1955) and *in vitro* (Sprott and Maclagan, 1955; Larson, Tomita and Albright, 1955).

The conjugation of thyroxine (T_4) and 3 : 5 : 3′-triiodothyronine with glucuronic acid was demonstrated in rat liver (Taurog, 1954; Roche *et al.*, 1954*b*), this process participating in the regulation of blood iodothyronines and their excretion.

Finally, modifications of the chemical nature of the benzene ring substituents accompanying or not the deiodination, and the eventuality of the breaking of the ether linkage have not yet been considered. The purpose of this work is to report and discuss the results we obtained in this field; the mechanisms proposed may constitute a model for an eventual metabolism of thyroid hormones *in vivo* and possibly lead to compounds acting without lag time.

The fact that biosynthesis as well as metabolism of thyroid hormones is always realized in aerobic conditions incited us to study the action of oxidizing enzymes on thyronine and its iodinated derivatives. As they are phenols we selected polyphenol oxidase which catalyses, specifically, oxidation of numerous phenols such as tyrosine

Material and Methods

Polyphenol oxidase was prepared by Kertesz and Zito (to be published) from "Champignon de Paris" (*Psalliota campestris*) by successive fractional extractions and precipitations with acetone, ethyl alcohol and ammonium sulphate—1 ml. of the enzymic solution contains 11·07 mg. of non-dialysable material, corresponding to 91,600 units, i.e. a specific activity (S.A.) of 8,270. The enzymic unit is defined by Hogeboom and Adams (1942) as the quantity of oxygen consumed in 1 minute by 1 mg. of 3 : 4-dihydro-L-phenylalanine (DOPA) in fixed conditions of dilution, temperature and agitation. 0·005 ml. of the preparation can completely oxidize 1·37 mg. tyrosine.

All substrates used for this work were purified by column or paper chromatography. Their analytical, spectrophoto-

enzyme catalyses the splitting of a part of the molecules used with the formation of tyrosine and small quantities of DOPA (Fig. 2). That phenomenon was investigated in its quantitative aspect. In the case of pigments A and C, for 4

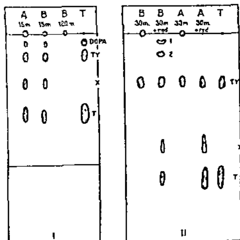


FIG 2 Schematic representation of chromatograms of medium containing pigments A and B

I. A 15 m —oxidation of thyronine after 15 min at pH 5 8, using the same enzyme/sub-

paper.
n-Butanol . acetic acid · water (78 : 5 : 17)
f = solvent front.

II. A 30 m —oxidation of thyronine after 30 min at pH 5 8
A 30 m + red—the same experiment after reduction for 30 min by sodium hydrosulphite
B 30 m and B 30 m + red—same experiment at pH 6 8
Whatman No 4 paper The solvent was allowed to flow for some hours after it attained the lower edge of the paper sheet

Results

Action of polyphenol oxidase on thyronine (Lissitzky, Bouchilloux and Kertesz, 1956)

The oxidation of thyronine by polyphenol oxidase at pH 5·8 in distilled water, pH 6·8 in 0·1 M phosphate buffer

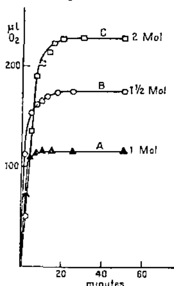


FIG 1. Oxygen consumption during oxidation of DL-thyronine by polyphenol oxidase at 3 different pH's: pH = 5 8 (pigment A); pH = 6 8 (pigment B); pH = 11 5 (pigment C) The main com-

water (A and C) or 0 1M phosphate buffer (B) (final volume 3 ml) Side arm of each flask contains 0 005 ml enzyme.

and pH 11·5 in dilute sodium hydroxide leads to the formation of three different pigments (A, B, C) which have been characterized by their absorption spectra in ultraviolet and visible light; the consumption of oxygen is respectively 1, 1·5 and 2 molecules O_2 per mole thyronine (Fig. 1).

A study of the products of the oxidation shows that the

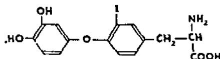
Kertesz and Lissitzky, 1956). Its physical, chemical and chromatographic properties were studied and the compound was undoubtedly identified with the product from enzymic oxidation. The pigments formed at acid and alkaline pH are of quinonoid nature, for gentle reduction regenerates thyronine and 3′-hydroxythyronine.

By using the property of *o*- and *p*-benzoquinones of forming fluorescent coloured complexes with *o*-phenylenediamine which can be characterized by their absorption spectra in visible light (Stein and Weiss, 1951), we have been able to show lately that the phenolic ring was probably changed during the oxidation of the thyronine by a mixture of 4-hydroxy-1·2-benzoquinone and 2-hydroxy-1.4-benzoquinone (unpublished results).

Action of polyphenol oxidase on iodothyronines

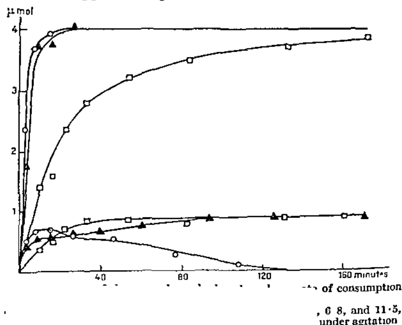
The oxidation of 3-monoiodothyronine was studied under the same conditions of substrate concentration as for thyronine. A red pigment appears at pH 7·0. The chromatographic investigation of the products formed shows that there is no deiodination but a breakdown of the ether linkage with liberation of monoiodotyrosine (Fig. 4). Part of the iodine is still contained in the pigment which does not migrate in the solvents which were used.

Mild reduction with sodium hydrosulphite regenerates 3-monoiodothyronine, with disappearance of the pigment. We also observed on the chromatograms of the incubation medium in *n*-butanol-acetic acid, a compound of R_F intermediate between that of 3-monoiodothyronine and that of monoiodotyrosine, being detected by ninhydrin, ammoniacal silver nitrate and ceric sulphate-sodium arsenite reagents. This compound has been identified as 3′-hydroxy-3-monoiodothyronine.



moles of thyronine oxidized, one mole of tyrosine is formed; the rate of formation of the latter seems to be slower than the rate of disappearance of thyronine (Fig. 3).

At pH 6·8 the concentration of the tyrosine rises to a maximum in 10 minutes, which corresponds to the consumption of all the thyronine; then its concentration falls and the tyrosine disappears altogether in 120 minutes.



of consumption

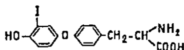
, 6 8, and 11·5, under agitation

1·5

38, 35

It has been possible to characterize the 3′- hydroxythyronine as an intermediate derivative in the oxidation of thyronine. *This compound* accumulates at acid pH but it is rapidly consumed at neutral and alkaline pH. The ascorbic acid, oxygen and Fe^{++} system of Udenfriend *et al.* (1954) is equally able to hydroxylate thyronine in the *o*-position; it has been used to prepare 3′-hydroxythyronine in pure form (*Bouchilloux,*

demonstrated by comparing the chromatogram in tertiary amylol saturated with $2N$-NH_4OH with that in phenol



I

II

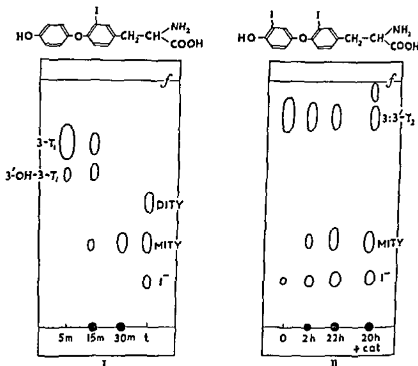
f = solvent front

saturated with water in an atmosphere of HCN, which constitutes a reducing solvent (Fig. 5).

The significance of these facts with regard to the mechanism of oxidation will be discussed later.

The oxidation of *3 : 3′-diiodothyronine* by the enzyme

3′-Monoiodothyronine under these conditions of substrate concentration is practically unoxidized. 8×10^{-5} M solutions



... (3-T₁) by polyphenol oxidase.

...ase.
...hol.

must be used to obtain an enzymic action. The total consumption of the substrate in 18 hours is then observed together with the conc[illegible] of iodides and of an iodinated pigment [illegible] on reduction. The quinon[illegible] easily be

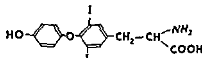
not attacked at concentrations under 2×10^{-5}M. 3 : 5-Diiodothyronine is particularly interesting; it is not oxidized at a concentration of $1 \cdot 66 \times 10^{-3}$ M but at $8 \cdot 3 \times 10^{-5}$ M it leads to the formation of diiodotyrosine and iodides in small quantities. Its oxidation by the ascorbic acid-oxygen-Fe^{++}

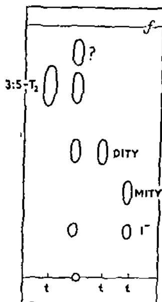
Table I

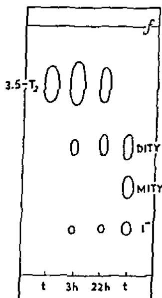
Substrate	*Molar concentration*	µg. *Substrate for 1 µl. enzyme*	*Oxidizability by enzyme*	*Halogen liberation*	*Other compounds formed*	*Breaking of the ether linkage*
Tyrosine	$2 \cdot 66 \times 10^{-3}$	274	+++		melanins	
MClTY	$2 \cdot 66 \times 10^{-3}$	210	++	+	melanins	
MBrTY	$2 \cdot 66 \times 10^{-3}$	260	+	+	melanins	
MITY	$0 \cdot 1 \times 10^{-3}$	19	±	+	?	
DClTY DBrTY DITY	$1 \cdot 66 \times 10^{-4}$	20	0			
Thyronine	$1 \cdot 66 \times 10^{-3}$	300	++++	+	pigments, TY, 3′-OH-T	+
3′-T_1	8×10^{-5}	5 8	++	+	iodinated pigment	+
3-T_1	$1 \cdot 66 \times 10^{-3}$	200	+++		iodinated pigment, MITY	+
3 : 3′-T_2	2×10^{-5}	0 01	+	+	iodinated pigment, MITY	+
3 · 5-T_2	$8 \cdot 3 \times 10^{-5}$	10	+	+	iodinated pigment, DITY	+
3′ 5′-T_2	8×10^{-5}	15	0			
3 5 : 3′-T_3	$3 \cdot 8 \times 10^{-5}$	0 25	0			
3 , 3′ ; 5′-T_3		0 10	?			
3 · 5 : 3′ . 5′-T_4	$2 \cdot 9 \times 10^{-5}$	0 25	0			
TRIAC and TETRAC	$1 \cdot 3 \times 10^{-5}$	10	0			

TY—tyrosine; MClTY, MBrTY, MITY—monochloro-, monobromo-, and monoiodotyrosine DClTY, DBrTY, DITY—dichloro-, etc. 3′-OH-T—3′-hydroxythyronine T_1, T_2, T_3, T_4,—mono-, di-, tri-, and tetraiodothyronine.

occurs only at concentrations about 2×10^{-5}M and a ratio of substrate/enzyme = 0·01 μg./μl. It leads to the release of







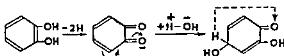
scorbic acid-

3 ml. 8 3 ×
5μl enzyme.

iodides and monoiodotyrosine, as well as the formation of a pigment (Fig. 4). The percentage of oxidized molecules is low as it does not exceed 10 per cent. It is increased by the addition of traces of catechol to the incubation medium.

3′:5′-Di-, 3:3′:5′-triiodothyronine and thyroxine are

genation reaction with the addition of the elements of water (in Gero, 1952).



However, the oxidation of thyronine requires a first stage of hydroxylation ortho to the diphenol group, followed by a dehydrogenation of the *o*-diphenol group formed. The necessity of a free ortho-position so that the oxidation might be accompanied could therefore be foreseen.

The study of the iodinated derivatives of thyronine shows that those which possess *at least* a free position in 3′ or 5′, are the only ones to be oxidized by the enzyme (3- and 3′-mono-iodothyronine, 3 : 3′- and 3 : 5-diiodothyronine). Yet 3 : 5 : 3′-triiodothyronine, which fulfils this condition, is not attacked, which seems to indicate the further necessity of an unsubstituted 3 or 5 position. This last limitation is only valid for our experimental conditions (370 enzymic units for 1 µg. 3 : 5 : 3′-triiodothyronine). It is not impossible that at a higher ratio enzyme/substrate it might be oxidized.

3:5-Diiodothyronine, whose 3 and 5 positions are substituted by iodine, is oxidized with the splitting of the ether linkage provided the ratio enzyme/substrate is high enough (10 enzyme units/µg.).

The oxidation of halotyrosines shows that, in the case of iodothyronines, the ortho-disubstituted derivatives are not oxidized. On the contrary, ortho-monohalogen derivatives (monochloro- and monobromotyrosine) are attacked though with a different velocity according to the nature of the halogen. The higher the molecular weight, the greater the velocity (Fig. 8). A concentration twenty-five times lower is necessary to obtain an enzyme action with monoiodotyrosine. Oxidation of monochloro- and monobromotyrosine leads to the formation of a fine red or purple pigment which rapidly turns into typical melanins. Cl^-, Br^- and I^- were found in the incubation medium. As in the case of iodothyronines the

system under the same conditions of substrate concentration produces the same compounds but in higher yield, and an unknown iodinated substance of high R_F value in butanol-acetic acid (Fig. 6). The results, summarized in Table I, correlate the number and position of the iodine atoms in the molecule with the oxidizability of the compound by the enzyme.

Discussion

The action of polyphenol oxidase on thyronine leads to the splitting of the ether link joining the two benzene rings of the amino acid with the formation of tyrosine. A part only of the

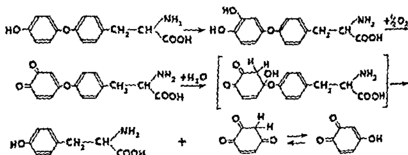


FIG 7.

molecules undergoes this kind of reaction. This process probably corresponds to the spontaneous hydrolysis of the ether linkage of the quinone corresponding to 3′-hydroxythyronine, the specifically enzymic phenomenon chiefly consisting in the oxidation of the o-diphenol (Fig. 7). This interpretation agrees with the following facts:

1. The purity of the enzyme preparation used (homogeneous with respect to electrophoresis and ultracentrifugation) makes the hypothesis that it could be a mixture of enzymes (oxidase + hydrolase) most unlikely. On the other hand it has never been proved that such preparations do possess hydrolytic activity.

2. The ordinary mechanism of o-diphenol oxidation, for example cathechol, implies the combination of a dehydro-

action of polyphenol oxidase on the monohalogenated derivatives of tyrosine implies a dehalogenation, the velocity of which varies with the nature of the halogen. The deiodination which goes with the oxidation of 3′-monoiodothyronine leads only to the release of about 50 per cent of the iodine contained in the latter, the remaining 50 per cent left being in the pigment, the structure of which corresponds to a quinonoid

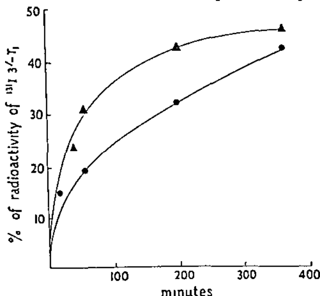


FIG 10. Oxidation of ^{131}I-labelled 3′-monoiodothyronine. Formation of iodides and iodinated pigment.
—●— : iodides.
—▲— : iodinated pigment.

form (Figs. 9 and 10). These facts allow us to propose the following stages for the oxidation of *o*-monohalophenols by polyphenol oxidase: the influence of the para substitution (R) remains to be specified (Fig. 11).

The summing up of these reactions emphasizes that the corresponding *o*-diphenol can be obtained together with a semi-quinonoid compound which appears to represent the pigment. Hydrogen iodide is formed in 50 per cent yield. The investigation of the oxidation of monochloro- and

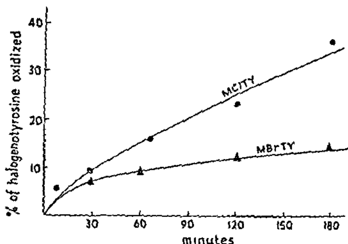


FIG. 8. Rate of oxidation of *monochlorotyrosine* (MClTY) and monobromotyrosine (MBrTY) by polyphenol oxidase 1·5 ml. 0 00266M solution in 0 1M phosphate buffer pH 6 7 + 0 004 ml. *enzyme. 37°, agitation in air.*

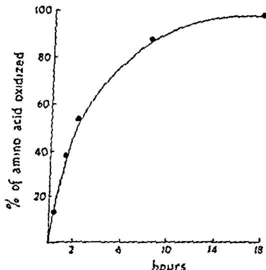


FIG. 9 Oxidation of ^{131}I-labelled 3′-monoiodothyronine by polyphenol oxidase expressed in % *disappearance* of the iodinated amino acid as a function of time

0 4 ml. 3′-monoiodo*thyronine* (11·8 μg., S A.—0·27 $\mu c/\mu g$) in 0 1M phosphate buffer pH 7·0 + 0 002 ml. enzyme.

hypothesis: the dehalogenating system of the thyroid glands acts equally on bromotyrosines and iodotyrosines (Roche *et al.*, 1953). All the dehalogenating systems are inhibited by the exclusion of oxygen or by the addition of inhibitors of oxidation sequences. Finally, the dehalogenating activity of different tissues seems to be linked at least to the particulate structure.

Some chemical systems, such as ascorbic acid-oxygen-Fe^{++} mixture under conditions compatible with cellular life (37° C—pH near neutrality—oxidizing medium), can bring about the deiodination of iodothyronines and especially of thyroxine and 3 : 5 : 3′-triiodothyronine (unpublished results). On another hand the action of polyphenol oxidase on certain iodothyronines and on thyronine itself leads to the formation of iodinated *o*-diphenols. This action, which may appear as a specific one of this enzyme, could be brought about, in theory, by other enzymes such as peroxidases, the distribution of which is widespread in animal tissues.

If we consider the possible mechanisms of deiodination we can propose three types of reactions (Fig. 12). From a consideration of the facts already proved, the reaction (3) is the most likely; reaction (1) must be rejected. Whether it is accomplished through reactions (2) or (3), the deiodination must lead to an *o*-diphenol, iodinated or not. We emphasize the eventual importance of such derivatives and their possible participation as active or activated hormones Their identification as unknown spots on chromatograms from tissue extracts of ^{131}I-injected animals is being investigated.

3 : 3′-Diiodothyronine, which must be regarded as a thyroid hormone (Roche, Michel, Truchot and Wolf, 1956), is oxidized by polyphenol oxidase with the release of iodine and monoiodotyrosine. As it appears to be a metabolite of 3 : 5 : 3′-triiodothyronine, the question is whether it is an obligatory product of the catabolism of thyroxine and 3 : 5 : 3′-triiodothyronine, which would throw some light on the deiodination of this hormone *in vivo*.

Polyphenol oxidase oxidizes neither diiodotyrosine, a

monobromotyrosine has not yet been achieved. An oxidative enzyme like polyphenol oxidase is thus able to catalyse indirectly the dehalogenation of o-monohalophenols. The same is true for Udenfriend's system (Udenfriend *et al.*, 1954)

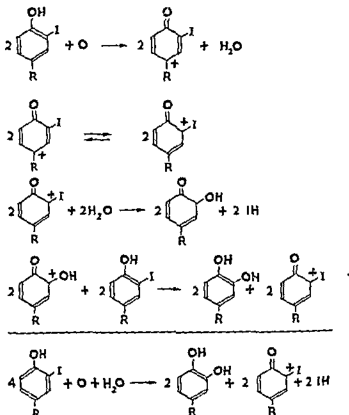


Fig. 11.

which fulfills at least two of the typical actions of the enzyme—the dehalogenation and the splitting of the ether linkage of thyronine structure.

This brings us to hypothesize on the deiodinating systems *in vivo.* … probably act as oxidativ… …m such as that mentione… …s bear out this

LISSITZKY, S., and ROQUES, M. (1956). *C.R. Soc. Biol., Paris*, **150**, 536.
ROCHE, J., MICHEL, O., MICHEL, R., and LISSITZKY, S. (1953). *C.R. Soc. Biol., Paris*, **147**, 232.
ROCHE, J., MICHEL, O., MICHEL, R., and TATA, J. (1954*a*). IInd Radioisotope Technical Conference, **1**, 325. Oxford University Press.
ROCHE, J., MICHEL, O., MICHEL, R., and TATA, J. (1954*b*). *Biochim. biophys. Acta*, **13**, 471.
ROCHE, J., and MICHEL, R. (1955). *Physiol. Rev.*, **35**, 602.
ROCHE, J., MICHEL, R., and JOUAN, P. (1956) This Colloquium, p 168.
ROCHE, J., MICHEL, R, TRUCHOT, R., and WOLF, W. (1956). *Biochim. biophys. Acta*, **20**, 337.
ROCHE, J., MICHEL, R., and WOLF, W (1954) *C R. Acad. Sci., Paris*, **239**, 597.
SPROTT, W. E., and MACLAGAN, N F (1955). *Biochem. J*, **59**, 288.
STEIN, G, and WEISS, J (1951) *J chem. Soc.*, 3265.
TAUROG, A. (1954) Brookhaven Symposia in Biology, No. 7, p 111.
UDENFRIEND, S., CLARK, C T., AXELROD, J., and BRODIE, B. B. (1954). *J. biol. Chem* **208**, 731
YAGI, Y, MICHEL, R., and ROCHE, J (1953) *Ann. pharm. franç*, **11**, 30.

DISCUSSION

[illegible]

triiodothyronine, for example, on a chromatogram?

Lissitzky This compound, in *n*-butanol-acetic acid, is expected to [illegible]

	Thyronine	3-Hydroxythyronine
n-Butanol-acetic acid-water (78 . 5 15) . . .	0 68	0 51
Ethyl alcohol-water (80 20) .	0 79	0 68

Taurog If I understood you correctly, this polyphenol oxidase does not act on thyroxine or on triiodothyronine, but does act on 3 · 3′-diiodothyronine

Lissitzky That is right

Taurog Do you postulate then that it has physiological significance in the periphery of the mammal in the deiodination process?

Lissitzky · No, I do not know. I give those results only as a model of

compound naturally deiodinated in aerobic medium by several tissues (thyroid, kidney, liver, intestinal mucosa), thyroxine nor 3 : 5 : 3′-triiodothyronine. In spite of this, we think that the study of its action has directed the investigation on the metabolism of iodothyronines towards the study of

(1) [OH, I, R-substituted benzene ring] + H_2 → [OH, H, R-substituted benzene ring] + IH

(2) [OH, I, R-substituted benzene ring] + H_2O → [OH, OH, R-substituted benzene ring] + IH

(3) 4 [OH, I, R-substituted benzene ring] + $\frac{1}{2}O_2$ + H_2O → 2 [OH, OH, R-substituted benzene ring] + 2 [O, I, R-substituted ring] + 2 IH

FIG 12.

other oxidative enzyme systems, which will help to determine the actual nature and mechanism of deiodination reactions in animals.

REFERENCES

... 27, 253

... 50). C.R. Soc.

... , 214, 709.

... York: Blakis-

... , N.Y., 76, 686.

... rm, 145, 273.

... nology, 56, 127.

... , E. C., (1956).

... Endocrinology, 57, 338.

LISSITZKY, S., BOUCHILLOUX, S., and KERTESZ, D. (1956). Bull. Soc. Chim. biol., Paris, 38, 35.

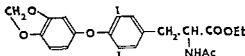
oxidation of 3 : 5-diiodothyronine by chemical and enzymic methods. You say that your compound had no thyroid activity?

Lardy: No We really should expect it to be inactive, for diiodothyronine, which might be a precursor for the dihydroxy compound, is not active.

P.-Rivers: I have thought for some time that hydroxylation of triiodothyronine in the 5′-position might lead to an active compound However, attempted hydroxylations by Udenfriend's method have not met with success, mainly because of solubility problems

Lissitzky: I have tried to prepare this compound that Dr Pitt-Rivers tried, but the products obtain by Udenfriend's method are very numerous. It is certain that this method does oxidize thyroxine and triiodothyronine, but we have not been able to identify with certainty the products formed. Dr. Pitt-Rivers, would it be possible to obtain this compound with diazotized triiodothyronine or thyroxine? I saw that a Belgian worker has published some work on diazotized thyroxine. Would it not be possible by hydrolysis of the diazotized triiodothyronine to obtain this compound in good condition?

P.-Rivers I think so, yes.



That compound will undergo hydrolysis in cold alkali to give the free acetamido acid leaving the methylene bridge intact. If more drastic conditions are tried, a dirty brown substance which loses iodine results The harder we tried to break down those protecting groups the more iodine was lost.

Lardy: We used the Glaxo route also, except that we used the dimethoxy compound. It hydrolyses quite cleanly without decomposition products.

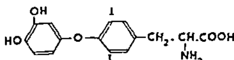
reaction; but we are now investigating the action of mammalian tyro- [illegible] from Hard... Passey melanoma of mice on those substrates, and [illegible]

[illegible] acts on monoiodotyrosine.

[illegible] the thyroid, do [illegible] ...ediate [illegible] to see [illegible] but I feel that many spots shown on radioautographs in many experiments in acidic solvents could possibly be of polyphenolic and polyiodinated nature. It is only an hypothesis; I have no evidence really that they can exist *in vivo* in animals.

Lardy: With reference to the schematic deiodination of thyroxine; the system which Larson and Albright discovered in kidney slices [illegible] so that we must consider an

[illegible] s been a very beautiful [illegible] ...ed here,



[illegible] we use: BMR in rats, [illegible] Unfortunately it is

homogenate has been found to convert thyroxine to other unknown compounds as is shown in Figure 1a. The radioactivity at the position occupied by iodide was present at zero time and did not increase during the incubation. No

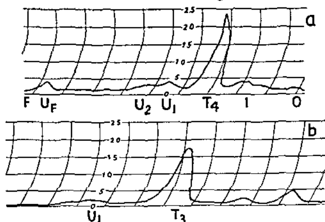


FIG. 1. Metabolism of thyroxine and triiodothyronine by rat kidney homogenate

[illegible]

...indicated by U_1, U_2, U_F and I respectively O and F indicate the origin and front of the chrom[illegible]

[illegible]

shown

(From Tomita, Lardy, Larson and Albright, 1956.)

detectable amounts of triiodothyronine were formed. Triiodothyronine was converted to an unknown which migrated faster than its parent compound and to two other more slowly moving substances (Fig. 1b).

When the kidney homogenate (in 0·25M sucrose) was

THE METABOLISM OF THYROID HORMONES BY KIDNEY AND THE BIOLOGICAL ACTIVITY OF THE PRODUCTS

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STUDIES of the mechanism of action of the thyroid hormone have been hampered by a lack of information concerning the chemical nature of the cellularly-active form of the hormone. For many years thyroxine was the only naturally-occurring form of the hormone available for *in vitro* experimentation. Now, as a result of the work of Gross and Pitt-Rivers and of Roche and Michel, we know that triiodothyronine is a naturally occurring form of the hormone with even greater potency than thyroxine. Thyroxine can be converted to triiodothyronine in the thyroidectomized rat (Gross and Leblond, 1951) or in human subjects (Pitt-Rivers, Stanbury and Rapp, 1955) and there is some evidence which indicates that this conversion may be an essential step in forming the "active" hormone. The conversion of thyroxine to triiodothyronine by surviving tissue *in vitro* was first demonstrated by Albright, Larson and Tust (1954). They demonstrated the reaction in kidney slices; some other tissues tried produced some iodide but did not accumulate a significant amount of triiodothyronine. Because of the possibility that the kidney plays a major rôle in converting thyroxine to a more active hormone a more detailed study of thyroxine metabolism by this organ has been undertaken.

Although the surviving kidney slice can deiodinate thyroxine to triiodothyronine, homogenates of this organ do not (Tomita, Lardy, Larson and Albright, 1956). However, the

triiodothyronine (Tomita *et al.*, 1956). Homogenates or mitochondrial preparations lost activity towards both thyroxine and triiodothyronine when heated to 100°. The enzyme system which brings about this conversion of thyroxine (T_4) and triiodothyronine (T_3) has been obtained in a completely soluble form by disrupting the mitochondria with sonic

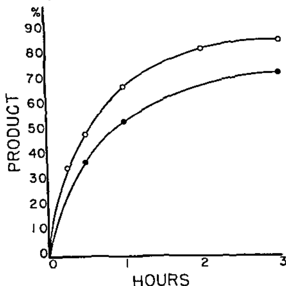


FIG. 3. Rate of formation of T_4U_1 (solid dots) and T_3U_1 (open circles) by rat kidney mitochondrial extract.
The reaction mixtures contained 26 ml dialysed extract of mitochondria from 7 g kidney, 2 m-moles phosphate buffer pH 7 4, 12 μmoles ATP, 10 μmoles

oscillation. Treatment for 8 minutes in the 10 KC Raytheon oscillator liberates the enzymes from the particles and the entire activity remains in solution after centrifugation for one hour at 105,000 *g*. Figure 3 demonstrates that the soluble enzyme preparation converts thyroxine and triiodothyronine to their respective unknowns 1 at about the same rate.

centrifugally fractionated according to Schneider (1951), the microsomes and soluble fractions were found to produce only small amounts of unknowns 1 and 2 from thyroxine (Fig. 2).

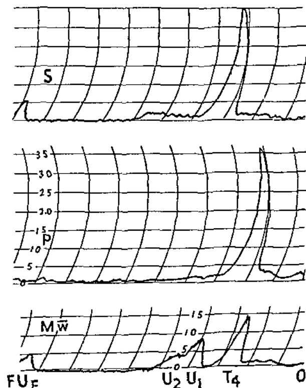


Fig. 2. Metabolism of thyro[illegible]

The mitochondrial fraction was found to be more effective in catalysing this conversion than was the whole homogenate. The fractions exhibited the same relative activities toward

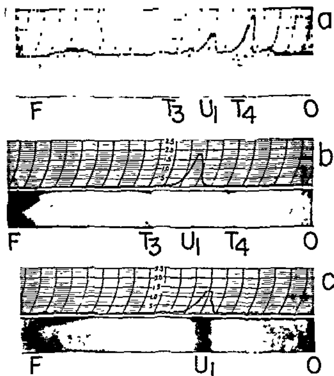


Fig. 4 Isolation of T_1U_1 and cochromatography with tetraiodothyroacetic acid

a Aliquot of reaction products with thyroxine (T_4) and triiodothyronine (T_3) added as reference compounds

[illegible]

c Position of labelled unknown corresponds with colour band of carrier tetraiodothyroacetic acid

Since appreciable amounts of free iodide were not produced and since we could recover in U_1 as much as 70 to 90 per cent of the radioactivity of the original hormone, it can be concluded that the unknowns contain as much iodine as their precursors. We have therefore designated them as T_4U_1 and T_3U_1.

If the solubilized enzyme preparation is dialysed, the rates at which thyroxine and triiodothyronine are converted are approximately doubled by the addition of diphosphopyridine nucleotide (DPN). Triphosphopyridine nucleotide and nicotinamide were ineffective. Succinate and other Krebs cycle intermediates enhance the activity of the dialysed preparation in the absence of DPN but not in the presence of the latter. Adenosine triphosphate was inhibitory under all conditions tested.

Identification of the Major Products

To obtain sufficient quantities of the unknowns for chemical characterization, experiments were conducted with 2 mg. of thyroxine or triiodothyronine as the substrate together with 25 ml. of dialysed enzyme in a final volume of about 50 ml. The reaction mixture contained also 2 m-moles of phosphate buffer pH 7·4, 10 μmoles DPN and 20 μmoles of succinate. The incubation period was 3 hours at 37°. The chromatogram of the reaction products from thyroxine are shown in Fig. 4a. 50 μg of non-isotopic thyroxine and triiodothyronine were added to the chromatogram and their position after migration is indicated on the strip by the colour formed on treatment with diazotized sulphanilic acid. The T_4U_1 peak was cut from other strips and rechromatographed as shown in section b, again with T_3 and T_4 as reference compounds. As can be seen, the isolated unknown gives a positive diazo reaction indicating the presence of a free phenolic group. Other samples gave a positive Kendall's test, indicating an *activated* phenolic group The ninhydrin test was *negative, suggesting* the absence of the α-amino acid side-chain. The compound did not react with 2 : 4-dinitrophenylhydrazine or semicarbazide (Umbreit

and Tonhazy, 1951) indicating the absence of a carbonyl function. It did not react with picric acid (Roche *et al.*, 1955) nor with *o*-phenylenediamine (Klitgaard *et al.*, 1953) indicating that it was not an α-keto acid.

The chromatographic behaviour of a great variety of analogues of thyroxine has been compared with T_4U_1. Two of the compounds tested, tetraiodothyroacetic acid (Harington and Pitt-Rivers, 1952) and tetraiodothyropropionic acid (Clayton, Green and Hems, 1951) could not be satisfactorily separated from T_4U_1 in four different solvent systems. With tertiary amyl alcohol saturated with 2N-NH_4OH the R_F values were: thyroxine 0·21, tetraiodothyropropionic acid 0·29, and tetraiodothyroacetic acid 0·27. The other systems which failed to resolve these compounds were (a) butanol-N-HCl at volume ratios of 20 : 1; (b) collidine saturated with water and with NH_3 in the atmosphere; (c) butanol-acetic acid-water at volume ratios of 80 · 20 : 20.

Evidence that T_4U_1 is not tetraiodothyropropionic acid was obtained by recrystallizing a mixture of the authentic compound with radioactive T_4U_1 (Table I). The loss of radioactivity clearly establishes the non-identity of these two

Table I

COCRYSTALLIZATION OF T_4U_1 WITH TETRAIODOTHYROPROPIONIC ACID

	Counts/min /µg
Original Mixture	4·29
1st crystallization	3 10
2nd crystallization	2 60
3rd crystallization	1 03
4th crystallization	0 78

substances. We conclude from the colour tests, the paper chromatographic studies and from the non-identity with tetraiodothyropropionic acid that T_4U_1 is probably tetraiodothyroacetic acid. Unfortunately, we did not have sufficient of the latter for cocrystallization with T_4U_1.

Similar large-scale experiments with triiodothyronine yielded sufficient T_3U_1 to demonstrate a positive Pauly reaction (Fig. 5), a positive Kendall test and negative reactions

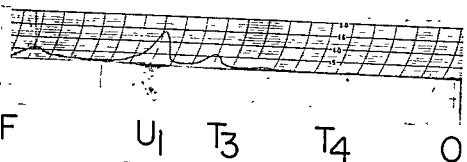


Fig. 5 Large-scale preparation of T_3U_1.
Conditions as described in text. 50 μg. thyroxine and 50 μg. triiodothyronine were added to the chromatogram Note the intense Pauly reaction given by the unknown.

Table III

BIOLOGICAL ACTIVITY OF THYROID HORMONES

	Tadpole	*BMR*	*Anti-Goitre*
HO–[$3{,}5$-I]C_6H_2–O–[$3{,}5$-I]C_6H_2–CH_2-$CHNH_2COOH$	1	1	1
R-CH_2CH_2COOH	21	0·1–0 2	0·2
R-CH_2COOH	+	+	0·5
R- COOH	0	0	0
R-$CH_2CH_2NH_2$	9	0 1	0 01
HO–[3-I]C_6H_3–O–[$3{,}5$-I]C_6H_2–CH_2CHNH_2COOH	5–10	5	10
R-CH_2CH_2COOH	4–15	0·6	0 3
R′-CH_2COOH	4–8	+	0 5
R′-COOH	0 1	0	0
R′-$CH_2CH_2NH_2$	36	0 1	0 1

+ Indicates activity but that it is not yet possible to give a quantitive value relative to L-thyraxine.

as possibly being the cellularly-active forms of the hormone. This point of view has been championed by Thibault and Pitt-Rivers (1955) and it has much to recommend it.

In Table III are shown the relative biological activities of TRIAC and TETRAC and related compounds as determined in our laboratory (Tomita and Lardy, 1956). The tadpoles used were *Rana clamitans*; the other two assays were performed with white rats. On a mole equivalent basis TETRAC and TRIAC are not as active as their parent thyronines. We must therefore continue the search for more active metabolic derivatives of iodinated thyronines or must find some new evidence that one or more of the known compounds participates in the reactions leading to the biological effects characteristic of the hormone.

REFERENCES

[illegible] *Proc Soc. exp.*
[illegible] *J. chem Soc ,*

with ninhydrin, 2 : 4-dinitrophenylhydrazine, semi-carbazide and picric acid. T_3U_1 migrated on paper in the four solvent systems described above at the same rate as triiodothyroacetic acid and only slightly faster than triiodothyropropionic acid. In the tertiary amyl alcohol system the R_F values were: triiodothyronine 0·40; diiodothyronine 0·46; triiodothyropropionic acid 0·50; and triiodothyroacetic acid 0·56.

When radioactive T_3U_1 was cocrystallized with authentic triiodothyropropionic acid (Tomita and Lardy, 1956) the radioactivity was rapidly lost (Table II). When mixed with

Table II

COCRYSTALLIZATION OF T_3U_1 WITH CARRIER COMPOUNDS

Carrier ⟶	*Triiodothyropropionic*	*Triiodothyroacetic*
	Counts/min./µg.	*Counts/min./µg.*
Original Mixture	8 45	3 39
1st crystallization	4 32	3 31
2nd crystallization	2 10	3·37
3rd crystallization	0 98	3 45
4th crystallization	—	3 53

triiodothyroacetic acid, the specific radioactivity remained constant through four crystallizations. This, together with the chromatographic behaviour of T_3U_1 and the qualitative chemical tests establish this compound as triiodothyroacetic acid.

Biological Activity

The demonstration of the enzymic formation of the tetra- and triiodinated thyroacetic acids (TETRAC and TRIAC, respectively) is of interest for several reasons. Roche, Michel and co-workers (1955) have found that radioactive triiodothyroacetic acid can be found in the kidneys of thyroidectomized rats treated with triiodothyronine containing ^{131}I. [illegible] accounts for the [illegible] produced by rats given [illegible]

and Pitt-Rivers, 1955; Trotter, 1955) they deserve attention

where oxidative phosphorylation is the rate-limiting step in respiration And in all tissues that have been studied with the possible exception of some tumours, the rate of phosphorylation is the limiting factor in determining the rate of respiration of the surviving tissue.

Thibault· So that you can have here an explanation of the action of TRIAC, TETRAC, or thyroxine and triiodothyronine This might be the very enzymic mechanism of action.

Lardy· We have always hoped that the *in vitro* demonstration of uncoupling was a counterpart of the situation in the whole animal where thyroxine causes an increased metabolism There are several questions that one has to ask First of all, is there a parallelism between compounds of different activity? Secondly, is that the way it functions under physiological conditions, or is that only the way it functions when there is an excess of the thyroid hormone? If the answer to the latter question is "Yes", does this explain the phenomena of thyrotoxicosis?

Taurog: Do you find this conversion of thyroxine and triiodothyronine to the corresponding acetic acid analogues to take place in any other mitrochondrial system than that of kidney?

know if this is accompanied, in the tadpole, by any perceptible increase in oxygen consumption?

Lardy· This has been studied by a great many workers and they disagree—at least there are two schools of thought. Some say the tadpole does increase its metabolism; others say it does not Those who say it does not, say it may on a dry-weight basis, but that if you calculate the rate of oxidation per tadpole there is no increase. We have never made any measurements so we cannot say. It is a very important question

Taurog The whole mechanism may be different in the tadpole from

Gross, J., and Leblond, P. (1951). *Proc. Soc. exp. Biol., N.Y.*, 76, 686.
Harington, C. R., and Pitt-Rivers, R. (1952). *Biochem. J.*, 50, 438.
Klitgaard, H. M., Lipner, H. J., Barker, S. B., and Winnick, T. (1953). *Endocrinology*, 52, 79.
Lerman, J., and Pitt-Rivers, R. (1955). *J. clin. Endocrin*, 15, 653.
Magasanik, B., and Umbarger, H. E. (1950). *J. Amer. chem. Soc.*, 72, 2308.
Pitt-Rivers, R. (1953). *Lancet*, 2, 234.
Pitt-Rivers, R., Stanbury, J. B., and Rapp, B. (1955). *J. clin. Endocrin. Metab.*, 15, 616
Roche, J., Michel, R., Jouan, P., and Wolf, W. (1955) *C. R. Acad. Sci., Paris*, 241, 1880.
Schneider, W. C. (1951). *In* Manometric techniques and tissue metabolism, p. 153., ed. Umbreit, W. W., Burris, R. H., and [illegible]
[illegible] ,95.
[illegible] C. (1956). *J. biol. Chem.* In press.
Trotter, W. R. (1955). *Lancet*, 2, 374.
Umbreit, W. W., and Tonhazy, N. E (1951). *Arch. Biochem. Biophys.*, 32, 96.
Wieland, T., and Fischer, E. (1949). *Naturwissenschaften*, 36, 219.

DISCUSSION

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TRIAC, TETRAC, or thyroxine and triiodothyronine. This might be the very enzymic mechanism of action.

Lardy: We have always hoped that the *in vitro* demonstration of uncoupling was a counterpart of the situation in the whole animal where thyroxine causes an increased metabolism. There are several questions that one has to ask. First of all, is there a parallelism between compounds of different activity? Secondly, is that the way it functions under physiological conditions, or is that only the way it functions when there is an excess of the thyroid hormone? If the answer to the latter question is "Yes", does this explain the phenomena of thyrotoxicosis?

Taurog: Do you find this conversion of thyroxine and triiodothyronine to the corresponding acetic acid analogues to take place in any other mitochondrial system than that of kidney?

Lardy: We have not done enough experiments with other tissues to give a definite answer We have done one experiment with liver but I should not like to be quoted on it. We have been so interested in what

in oxygen consumption?

Lardy: This has been studied by a great many workers and they disagree—at least there are two schools of thought. Some say the tadpole does increase its metabolism, others say it does not. Those who say it does not, say it may on a dry-weight basis, but that if you calculate the rate of oxidation per tadpole there is no increase. We have never made any measurements so we cannot say. It is a very important question

Taurog: The whole mechanism may be different in the tadpole from a mammal. You may be affecting different systems completely.

Lardy: Certainly And that is especially brought out by the fact that the adult frog does not respond to thyroid hormone. It can receive

extent of about 30 per cent of the thyroxine originally present. Why we do not find triiodothyronine and TRIAC more often produced, I have no idea.

[illegible]

It looks very much as though there would be a whole series of thyroactive compounds, some of which may be inter-convertible I cannot quite see how a propionic acid could be produced from an alanine [illegible]

Barker I tried to corner Dr. Tata on that but he did not seem quite sure whether it was really tetraiodothyropropionic or the acetic acid.

In our hands the tetraiodothyroformic, the one-carbon material, has slight metabolic activity, but interestingly enough the butyric has a considerable amount of activity so the curve does not fall off equally at both ends. I suspect that, if the thyroxine or the thyroactive material is fastening on anywhere by means of the carboxyl group, there has got to be a certain amount of clearance for the ring Apparently the C-1 [illegible]

Roche: We found, like you Dr Barker, some TETRAC in the kidney after injection of radioactive thyroxine into animals We tried to find if the presence of triiodothyronine could be shown by paper chromatography after the first results obtained by Larson and Albright with kidney slices And we found just at the spot where Larson and Albright found triiodothyronine, a mixture of TETRAC and triiodothyronine, but we were able to separate these by paper electrophoresis. So we completely agree with you

Lissitzky: Dr. Lardy, have you tried to obtain the formation of chelates with other iodothyronines than thyroxine and triiodothyronine? [illegible]

modification by substitution.

Lardy: We can confirm this double peak in the case of the diiodothyronines; it is very striking. At first I thought it was an error, but it really is there and I have no explanation for it

Lissitzky: It is a striking fact that when another atom of iodine is added, as in 3:5.3′-triiodothyronine compared to 3.5-diiodothyronine, there is no proper band.

Lardy: What we have thought is that possibly this meant that we are measuring the absorption of the individual rings there and that in the other compound you have enhanced absorption by the molecule which

this fine differentiation

Lissitzky: Yes, but when you study it this is very difficult for thyroxine, but for thyronine when you survey the spectrum in acidic and alkaline conditions you see that in acidic conditions you have two bands and only one in alkaline conditions. Did you find that also? And can you give an explanation of that?

Lardy: The only explanation I could give is that the phenolic group of thyroxine and triiodothyronine ionizes so much more readily than that of thyronine or diiodothyronine, at pH 7, for example, thyroxine is 90 per cent or so in the phenolate form whereas thyronine is still in the free phenolic form.

ON THE PRESENCE OF 3:5:3′-TRIIODOTHYROACETIC ACID AND 3:3′-DIIODOTHYRONINE IN RAT MUSCLE AND KIDNEY AFTER ADMINISTRATION OF 3:5:3′-TRIIODO-L-THYRONINE

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THE thyroid hormones (3 : 5 : 3′ : 5′-tetraiodo-L-thyronine or thyroxine, 3 : 5 : 3′-triiodo-L-thyronine, 3 : 3′ : 5′-triiodo-L-thyronine and 3 : 3′-diiodo-L-thyronine†) are metabolized in the organism along three principal pathways. First, a process of dehalogenation liberates the iodide, since this appears in the urine after injection of any one of the four iodothyronines (Joliot *et al.*, 1944; Roche, Michel and Tata, 1954*a*; Roche, Michel, Etling and Nunez, 1956). Second, they are glycuro-conjugated in the liver (Taurog, 1954; Roche, Michel, Michel and Tata, 1954; Roche, Michel, Etling and Nunez, 1956). And third, their oxidative deamination leads to the corresponding iodopyruvic acid (Roche, Michel and Tata, 1954*b*) without previous deiodination.

Since the formation of 3 : 5 : 3′ : 5′-tetraiodo- and 3 : 5 : 3′-triiodothyropyruvic acids in the liver and probably also in the kidneys has been established as coming from their hormonal precursors, it was important to look into their metabolic fate. The iodothyroacetic acids which could possibly originate from their oxidation seem to be of unusual interest. These recently synthesized substances show a fleeting (Pitt-Rivers, 1953) but immediate action on rat oxygen consumption (Thibault, 1956) and on rat kidney slices (Pitt-Rivers and Thibault, 1955; Thibault and Pitt-Rivers, 1955*a*), while the action of the hormones appears only after some length of time (Thibault,

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TRAC.

1955; Thibault and Pitt-Rivers, 1955*b*). The question then is to determine whether they are "the active form" of the thyroid-secreted hormones, or one of their physiologically more active metabolites. We undertook to study the products of the metabolism of 3 : 5 : 3′-triiodothyronine in the receptor tissues of thyroidectomized rats. We hoped to find among them deiodinated derivatives and substances derived from the oxidation of the alanine side-chain.

Experimental

Chromatographic methods

To detect the metabolic products of 3 : 5 : 3′-triiodo-L-thyronine which we wanted to identify in receptor tissues, we had to work out a method of which two special aspects should be mentioned. On the one hand, we defined the chromatographic and ionophoretic behaviour of the reference substances, which allowed us to proceed with preparations and characterizations. On the other hand, there were special technical problems involved due to the very small quantities of iodothyronines and their derivatives present in the tissues.

The chromatographic solvents best suited to our studies were *n*-butanol saturated with 2N-NH_4OH (descending) and *tert*.-pentanol saturated with 2N-NH_4OH (ascending). The R_F values of the various substances on Whatman No. 1 paper at 15° with the two solvents are given in Table I.

Ionophoresis on paper in 0·05 M ammonium carbonate buffer, pH 9, was a helpful addition to paper chromatography in our studies (using 12 v/cm length of paper and 1 mA/cm. width). The reference substances take up very different positions (as revealed by Pauly reagent for the iodothyronines and their derivatives and by ceric sulphate). This way, iodide can be eliminated by short ionophoresis and the other constituents of the extract, after elution, can be subjected to a separative technique. In five hours 3 · 3′-T_2 is 3·5 cm. from the starting-line, 3 : 5 : 3′-TRIAC and 3 : 5 : 3′ : 5′-TETRAC are 6 and 7·5 cm. respectively; T_4 and 3 : 5 : 3′-T_3 do not move.

Identification of these six iodinated compounds in a mixture can be carried out using either two-dimensional chromatography in the solvents indicated, or by coupling one-dimensional chromatography with ionophoresis at right angles. Both techniques have been applied under the best conditions after ionophoretic elimination of iodide and elution of the iodothyronines and their derivatives.

Table I

R_f Values of the Various Iodinated Derivatives

Substances	R_f *in n-butanol saturated by* 2N-NH_4OH 15°	R_f *in tertiary pentanol saturated by* 2N-NH_4OH 15°
Thyroxine (T_4)	0·48	0·28
3 : 5 : 3′-triiodothyronine (T_3)	0·65	0·46
3 : 3′-diiodothyronine (T_2)	0·51	0·36
3 : 5 : 3′ . 5′-tetraiodothyroacetic acid (TETRAC)	0·66	0·40
3 : 5 : 3′-triiodothyroacetic acid (TRIAC)	0·75	0·55
Iodide	0·31	0·18

3 : 5 : 3′-Triiodothyronine, labelled in position 3′, injected into animals required very high specific radioactivity in order to be able to study its metabolism after administration of physiological doses (0·10—0·25 μg.). It was prepared in quantities of around 15–20 μg., with a radioactivity of 5–10 mc, in accordance with a method we recently described (Roche, Michel, Jouan and Wolf, 1955).

Muscle

All experiments were carried out on male rats of about 180 g., thyroidectomized 15–20 days previously and kept at 25°. In our studies of the kidney the animals were killed four hours after intraperitoneal injection of a single dose of 3 : 5 : 3′-triiodo-L-thyronine (0·25 μg./50 μc per animal).

In the case of muscle, part of the experiments were carried out on animals having also received a single dose of the hormones (0·1 µg./30 µc). In other experiments, the same quantity of 3 : 5 : 3′-triiodothyronine was given in three injections, 10, 4, and 2 hours before killing by bleeding. The treatment of the ammoniacal extracts of the organs comprising new techniques has been described in detail elsewhere (Roche, Michel and Jouan, 1956). We will indicate here only the general procedure.

Almost all the skeletal muscle was cut out (220 g.), frozen, ground up in the presence of dry-ice and extracted by 1 per cent NH_4OH (800 ml.). The radioactive solution obtained (3 per cent of the total radioactivity injected) is lyophilized and the resulting powder (15 g.) delipidated by 500 ml. of a mixture of 95 per cent peroxide-free ether and 5 per cent absolute ethanol,* then deproteinized (taken up by 150 ml. 1 per cent NH_4OH, and ethanol added up to 65 per cent); these operations were carried out at 0° and under CO_2 in order to avoid any deiodination. The alcoholic solution is then concentrated *in vacuo* (under N_2, temperature $< 30°$) and taken up by 4 ml. 2N-NH_4OH and concentrated again *in vacuo* to a volume of 2 ml. A small aliquot of the ammoniacal concentrate was analysed by two-dimensional chromatography (Whatman No. 1 paper) using as solvent, first, *n*-butanol saturated with 2N-NH_4OH (descending), and second, *tert.*-pentanol saturated with 2N-NH_4OH (ascending). Three main radioactive spots could be found by autoradiography and their constituents identified respectively as 3 · 5 : 3′-T_3, 3 . 3′-T_2 and iodide (Fig. 1)

With them there is in general a much weaker spot and it could be supposed that it corresponded to 3 · 5 : 3′-TRIAC. We carried out a second experiment under conditions more favourable to the characterization of this substance.

Six rats were treated in the same way as in the first experiment except that 0·1 µg. of 3 : 5 : 3′-triiodo-L-thyronine

* TRIAC is extracted by ether from its acid solution, but this is not the case for its alkaline salt under the conditions of our experiments.

was given in three injections, 10, 4, and 2 hours before killing. The muscle ammoniacal extract obtained as indicated above was utilized to show the presence of TRIAC. In order to do this, it was subjected to one-dimensional preparative chromatography in the presence of *n*-butanol saturated with 2N-NH_4OH. The substances whose R_F correspond respectively to 3 : 5 : 3′-T_3 and 3 : 5 : 3′-TRIAC were eluted with water-saturated *n*-butanol. The eluate of the latter was analysed by ascending chromatography in *tert.*-pentanol saturated with 2N-NH_4OH, followed by electrophoresis at right angles (500 v, 20 mA, 5 hr.) in 0·05 M-$(NH_4)_2CO_3$ buffer. Two spots could be detected. One corresponds to 3 : 5 : 3′-T_3, the other to 3 : 5 : 3′-TRIAC (R_F in *tert.*-pentanol = 0·46 for 3 : 5 : 3′-T_3, and 0·53 for 3 : 5 : 3′-TRIAC). Mobility is 5 cm. for 3 : 5 : 3′-TRIAC and zero for 3 : 5 : 3′-T_3 under the conditions of ionophoresis used (Fig. 2). Two-dimensional chromatography, with the same solvents, of an eluate of the spot of R_F corresponding to 3 : 3′ : 5′-T_3 shows that this spot contains a mixture of 3 : 3′-T_2 and 3 : 5 : 3′-T_3 (Fig. 3).

The results obtained established the presence of iodide, of 3 : 3′-T_2 and of 3 : 5 : 3′-TRIAC together with 3 : 5 : 3′-T_3 in rat muscle after physiological injection of the latter.

Kidney

Groups of three male rats thyroidectomized 15 days previously were each given intraperitoneally 0·25 μg. T_3 labelled in position 3′ by 50 μc ^{131}I and killed 4 hours later. ...zen and ground up ... which is taken up ... acts contain about 2 per cent of the total radioactivity given. They are lyophilized and the product extracted five times by 10 ml. portions of an ether-alcohol mixture (95 per cent peroxide-free ether and 5 per cent absolute ethanol). Less than 1 per cent of the radioactivity passes into the ether phase. The dry lipid-free residue is taken up by 2 ml. water and then dissolved by slow addition of 1 ml. 2N-NH_4OH. The proteins precipitated by

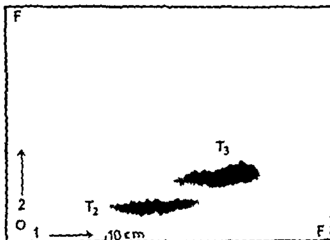
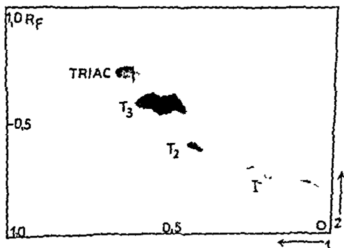
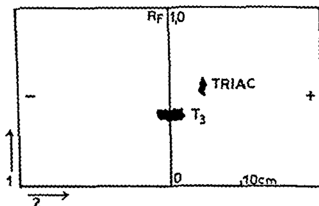


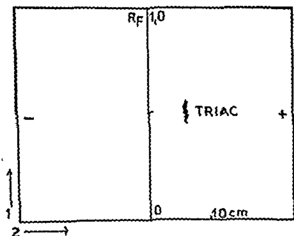
Fig. 3 Two dimensional chromatogram of the strip eluate at the level of 3:5-diiodothyronine and 3:5:3′-triiodothyronine.
1st dimension *n*-butanol saturated with 2N-NH_4OH
2nd dimension *tert*-pentanol saturated with 2N-NH_4OH



Fi [illegible]



(a)



(b)

FIG. 5 Chromato-electrophoregram (a) of the strip eluate at the level of triiodothyronine, (b) of the strip eluate at the level of TRIAC

1st dimension *tert*-pentanol saturated with 2N-NH_4OH,
2nd dimension: electrophoresis in 0.05M-$(NH_4)_2CO_3$ buffer, pH = 9

7·5 ml. of 95 per cent ethanol are eliminated by centrifugation. The supernatant is separated and a second extraction is carried out on the residue under the same conditions. The ammoniacal solution (18 ml.) contains 95 per cent of the radioactivity concentrated by the kidneys. 18 ml. chloroform and 6 ml. 2N-NH_4OH are added after centrifugation, the upper layer is removed, while the lower layer is extracted twice more by 6 ml. 2N-NH_4OH. 80 per cent of the initial radioactivity is recovered. All the operations are carried out at 0° under CO_2 in order to avoid any deiodination. The ammoniacal extracts are evaporated *in vacuo* and under nitrogen down to about 2 ml., and the concentrated solution obtained is placed on a strip of Whatman No. 1 filter paper and eluted with water-saturated *n*-butanol. The pigments present remain on the paper and the eluate contains practically all the radioactivity. An aliquot is analysed using two-dimensional chromatography, first with *n*-butanol saturated with 2N-NH_4OH. A radioautogram of this chromatogram is obtained by placing on it a film of Kodak Kodirex. Four main spots and several others of lower intensity are found. The position of these four spots corresponds to those of 3 : 5 : 3′-TRIAC, 3 : 5 : 3′-T_3, 3 : 3′-T_2 and iodide (Fig. 4). The areas of the chromatogram corresponding to the exact position of the radioactive spots found on the radioautogram are cut out and counted in a well-type scintillation counter; this enabled us to establish the percentage distribution of the various radioactive substances originating from the renal metabolism of 3 : 5 : 3′-triiodothyronine.

Compounds	*% Radioactivity*
3 : 5 : 3′-TRIAC	9·7
3 : 5 : 3′-T_3	78
3 : 3′-T_2	6·4
I^-	2

The spots of secondary intensity do not contain more than 4 per cent of the total radioactivity.

In order to confirm the existence of 3 : 5 : 3′-TRIAC, a further purification of the remaining total extract is carried out by preparative chromatography in *n*-butanol saturated with 2N-NH_4OH. After development two strips of R_F = 0·65 (T_3) and 0·75 (TRIAC) are cut out and eluted with water-saturated *n*-butanol. These two eluates are separately analysed using first *tert.*-pentanol saturated with 2N-NH_4OH followed by electrophoresis at right angles. The ionophoresis is carried out for 5 hours in 0·05 M-$(NH_4)_2CO_3$ at pH = 9 (12 v/cm. length and 1 mA/cm. width). The R_F of triiodothyronine in *tert.*-pentanol is 0·46 and that of TRIAC is 0·55. Under the conditions of electrophoresis triiodothyronine does not move while TRIAC moves 6·23 cm. The radiochromatoelectropherogram of the strip corresponding to triiodothyronine shows two radioactive spots, one of triiodothyronine and another, weaker, of TRIAC; the strip corresponding to TRIAC shows a single spot at the level of TRIAC reference (Fig. 5a and b).

Discussion and Conclusions

The significance of the observed facts should be discussed briefly as far as the formation of 3 : 3′-T_2 and 3 : 5 : 3′-TRIAC is concerned.

1. For a long time, the existence of tissue deiodination processes had been known but there was a special problem involved. Since 3 : 5 : 3′-triiodo-L-thyronine is five times more active than thyroxine, it had been supposed that it was its "active form" and that it originated by partial deiodination (Gross and Pitt-Rivers, 1953). Experiments on the subject have not given absolutely clear results. Those carried out *in vivo* have been done on non-thyroidectomized animals (Flock and Bollman, 1955); then their glands, reusing the labelled iodide from the degradation of the radioactive thyroxine injected, could have been the source of the triiodinated hormone present in the tissues. Those carried out *in vitro* with organ extracts did not permit a precise characterization

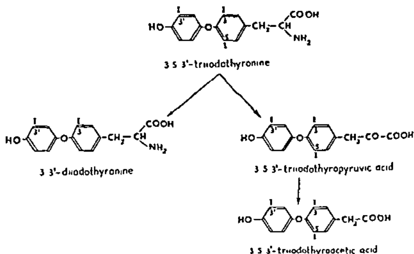
of the products of dehalogenation of L-thyroxine (Sprott and Maclagan, 1955). Only by having kidney slices act on solutions containing thyroxine could significant results be obtained, in the sense that a substance having the same R_F as 3 : 5 : 3′-triiodothyronine in one-dimensional chromatography appears in small proportions in the reaction medium.

However, 3 : 5 : 3′ : 5′-TETRAC has the same R_F as 3 : 5 : 3′-triiodothyronine in the solvent used, and it could be inferred from earlier results (Larson, Tomita and Albright, 1955) that it is probably formed from L-thyroxine under the experimental conditions. Unpublished studies allow us to identify under these conditions a mixture of TETRAC and of 3 : 5 : 3′-triiodothyronine. Anyway, the rapid formation of 3 : 3′-diiodothyronine from its triiodinated homologue seems to be *an important step in the metabolism of the latter;* 3 : 3′-diiodothyronine is found in kidney and muscle after injection of its precursor, and we also showed it to be present in egg-yolk after injecting 3 : 5 : 3′-triiodothyronine into laying hens (Roche, Michel and Volpert, 1956). It seems then that, if the deiodination of L-thyroxine proceeds by successive steps in their receptor cells, the formation of 3 : 5 : 3′-triiodo-L-thyronine does not have the hoped-for importance. It constitutes, if it actually happens, only an intermediate step of the dehalogenation of L-thyroxine to 3 : 3′-diiodothyronine. This product seems to be the ultimate one in the dehalogenation of the iodothyronines before their metabolic degradation by rupture of the ether linkage between the two benzene rings. It is one of the pathways of the catabolism of the iodothyronines, probably without any important connection with their physiological activity.

2 The formation of 3 5 : 3′-TRIAC has an entirely different meaning. In addition to dehalogenation, the metabolism of the hormone takes another direction at the same time as the alanine residue of 3 . 5 : 3′-triiodothyronine undergoes a degradation without removal of the iodine atoms on the benzene ring. As has been pointed out earlier, probably the

first step in this process leads to 3 : 5 : 3′-triiodothyropyruvic acid, which, in the second step, is decarboxylated to 3 : 5 : 3′-triiodothryoacetic acid. Since these substances are very diffusible the fact that they are found only in small quantities does not indicate that the reactions which give rise to them are not intense.

It should be pointed out that these results on muscle are much clearer than those on kidneys. The latter can present only a special type of metabolism of the triiodothyronines connected with a detoxification function, but they can also limit themselves to concentrating the 3 : 5 : 3′-TRIAC circulating in the blood in trace amounts. Unpublished studies enabled us to identify this substance as an unknown iodinated derivative excreted in the urine after injection of 3 : 5 : 3′-triiodothyronine. Anyway there is no doubt about there being two pathways for the metabolism of 3 : 5 : 3′-triiodo-L-thyronine, and the first step of both is in accordance with the scheme below.



The action of each of these two derivatives, the presence of which has been established in kidney and muscle after injection of 3 : 5 : 3′-triiodothyronine, is not equivalent as far

as the physiological effects of the product administered are concerned. It has been questioned for a long time whether the thyroid hormones have a direct pharmacodynamic effect or whether they act only through derivatives of their metabolism. Since 3 : 3′-diiodo-L-thyronine is six to seven times less active than 3 : 5 : 3′-triiodothyronine, the dehalogenation of this substance leads to a great reduction of its exectiveness, and should be considered as a simple degradation process. However, 3 : 5 : 3′-TRIAC acts immediately without any loss of time, and it is possible that the effects of the hormone are connected with the formation of this substance from its precursor. New studies are necessary to obtain a definite opinion on this subject. It would be helpful to formulate it to dispose of quantitative data on the transformation rate of 3 : 5 : 3′-triiodothyronine to 3 . 5 : 3′-TRIAC, and on the respective activities of these two substances.*

Summary

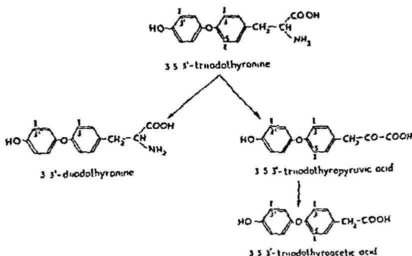
1. Muscle of thyroidectomized rats contains 3 : 3′-diiodo-L-thyronine and 3 : 5 : 3′-triiodothyroacetic acid, after injection of physiological doses of 3 · 5 : 3′-triiodo-L-thyronine (0·1 μg.). These results, together with our earlier ones on kidneys from rats subjected to the same treatment, show that the metabolism of the injected hormone can take two pathways: partial deiodination without change in the alanine residue, and degradation of this residue (oxidative deamination and decarboxylation, without any deiodination).

2 Since the action of 3 : 5 : 3′-triiodothyroacetic acid on the metabolic rate is immediate, while that of the thyroid hormones has a fairly long lag time, it could be asked whether the iodothyroacetic acids were not in their active form. Thus the proof of the existence of one of these iodothyroacetic

* [illegible]

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Barker: I do not see how we can tell at the moment, but with an effect as, for instance, on the succinoxidase system, you get an activity parallel to the presence of the 3:5-diiodo-4-hydroxyphenyl configuration
s some evidence
l up with iodo-

e-chain for the
uncoupling.

Lissitzky: I think that this discussion leads to the conclusion that

hormones, because it is not actually possible to see a determined point

metabolism.

Roche: I think the data presented yesterday by Prof Courrier show

mones of many other organs.

Lardy: I endorse what has been said if you look at it in our present state of knowledge, certainly many phenomena are affected, but I think this should not stop us from trying to find the exact chemical reactions which are influenced *in vitro* or eventually, if we can, *in vivo* I look forward to the time when we can write exact equations describing the mechanism of action, as we now can for the pathways of metabolism

acids in the receptor cells (muscle and kidney) gives to our studies a special importance since 3 : 5 : 3′-triiodothyroacetic acid had never been detected in these tissues before.

REFERENCES

FLOCK, E. V., and BOLLMAN, J. L. (1955). *J. biol. Chem.*, 214, 709.
GROSS, J., and PITT-RIVERS, R. (1953). *Biochem. J.*, 53, 645.
JOLIOT, R., COURRIER, R., HOREAU, A., and SUE, P. (1944). *C. R. Soc. Biol., Paris*, 138, 325.
LARSON, F. C., TOMITA, K., and ALBRIGHT, E. C. (1955). *Endocrinology*, 57, 338.
PITT-RIVERS, R. (1953). *Lancet*, 2, 234.
PITT-RIVERS, R., and THIBAULT, O. (1955). *C. R. Acad. Sci., Paris*, 240, 668.
ROCHE, J., MICHEL, O., MICHEL, R., and TATA, J. (1954). *Biochim. biophys. Acta*, 13, 471.
ROCHE, J., MICHEL, R., ETLING, N., and NUNEZ, J. (1956). *Biochim. biophys. Acta*, 19, 490.
ROCHE, J., MICHEL, R., and JOUAN, P. (1956). *Bull. Soc. Chim. biol.*, 38, 941.
ROCHE, J., MICHEL, R., JOUAN, P., and WOLF, W. (1955). *Bull. Soc. Chim. biol.*, 37, 819
ROCHE, J., MICHEL, R., and TATA, J. (1954*a*). *C. R. Soc. Biol., Paris*, 148, 1036
ROCHE, J., MICHEL, R., and TATA, J. (1954*b*). *Biochim. biophys. Acta*, 15, 500.
ROCHE, J., MICHEL, R., and VOLPERT, E. (1956). *C. R. Soc. Biol., Paris*, 150, 24.
SPROTT, W. E., and MACLAGAN, N. F. (1955). *Biochem. J.*, 59, 288.
TAUROG, A. (1954). Brookhaven Symposia in Biology, No. 7, p. 111.
THIBAULT, O. (1955). *C. R. Soc. Biol., Paris*, 149, 877.
THIBAULT, O. (1956). *Ann. Endocr., Paris.*, 17, 35.
THIBAULT, O., and PITT-RIVERS, R. (1955*a*). *Lancet*, 1, 285.
THIBAULT, O., and PITT-RIVERS, R. (1955*b*). *C. R. Soc. Biol., Paris*, 149, 880.

DISCUSSION

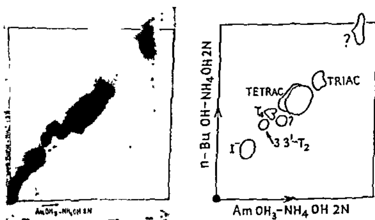


FIG 1 (Lissitzky).

thyronine I⁻ — iodides ? — unknowns

you get a much better separation of the amino acids and the acetic analogues with only normal or half-normal ammonia. Using ammonia in lesser concentrations the amino acids move more readily with the solvent front, and when you increase the ammonia concentration they remain in the first part of the chromatogram.

Lardy: Let me give you the R_F values in the tertiary amyl alcohol 2N ammonium hydroxide system.

Thyroxine	0·21
Tetraiodothyropropionic acid	0·29
Tetraiodothyroacetic acid	0 27
Triiodothyronine	0·4
Diiodothyronine	0·46
Triiodothyropropionic acid	0·5
Triiodothyroacetic acid	0·56

To effect better separation, Larson and Albright have sometimes used a chromatographic chamber that is four feet high.

Taurog: You mean the filter paper is four feet long?

Lardy: Yes.

Gross: If I may add a disturbing technical point. Recently we were checking injection mixtures with tertiary amyl alcohol-ammonia—these were presumably pure triiodothyronine—and it was very disturbing to us to see that when we did a two-dimensional chromatogram in tertiary amyl alcohol-ammonia we could demonstrate a rather lovely spectrum of [illegible]

acid with high activity and we have much data to show that the acetic analogue will be produced.

P.-Rivers On the paper?

Lissitzky: Perhaps on the paper or perhaps in the solution, because this process increases with time.

THE DISTRIBUTION AND METABOLISM OF THYROID HORMONES

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S. Symchowicz and J. H. Horton

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The distribution of thyroxine and triiodothyronine labelled with ^{131}I has been the subject of a continuing study. It has been found that while thyroxine is concentrated mainly in the liver and kidney, triiodothyronine is markedly concentrated by a wide variety of organs and tissues (Gross, 1955). This report represents part of a series of experiments designed to investigate the chemical nature of the concentrated radioiodine and the dynamics of its concentration.

Materials and Methods

Male albino rabbits obtained from a commercial breeder were injected intravenously with ^{131}I-labelled triiodothyronine (Abbott Laboratories, Oak Ridge, Tenn., U.S.A.). The usual dose administered was about 5 μg./kg., but in some experiments one-tenth this amount was used.

The animals were maintained under anaesthesia, and serial blood, muscle and bile samples were taken over a 4-hour period. At the end of the experiment a number of organs were also removed. In three experiments the bile was collected from the cannulated bile duct. In three experiments the bile duct was cannulated with a polyethylene tube and the other end was inserted into the distal portion of the bile duct. A loop of the tubing was brought out through the abdominal incision which permitted sampling of bile without completely

interrupting its flow into the intestine. In the final three experiments so far carried out there was no surgical intervention in the biliary system and at the end of the experiment the gall bladder bile was aspirated.

The samples were analysed by chromatographic methods (cf. Gross, 1954); the radioactivity was detected by either radioautography, or by scanning under an end window flow counter. Quantitative data were obtained by counting fractions in a well-type scintillation counter.

Results and Discussion

The results of all experiments were essentially the same and therefore a typical experiment will be used to illustrate the findings.

The injected radioactivity disappeared rapidly from the circulation (Fig. 1), some 75 per cent of the dose having left the circulation in 6 minutes. The percentage of the dose in the muscle mass increased at a much slower rate and its ^{131}I content could account for only a small proportion of the dose lost from the circulation during the same time period. This same relationship was obtained in a study on mice (cf. Gross, 1955) where it could be shown that primarily the liver, and the kidney to a much lesser extent, were responsible for clearing the circulation of triiodothyronine. In both studies, too, the increase in the proportion of the dose found in the musculature at later time intervals (e.g. after 1 hour) was greater than could be accounted for by the decrease in plasma content of radioactivity. This was interpreted as meaning that the injected material had passed through the liver and/or the kidney before it could enter the muscle.

The product from the liver and/or the kidney returning to the circulation should be a metabolite of triiodothyronine. Such a product is demonstrable in the circulation (Fig. 2, "x") and substances with the same chromatographic mobility are seen in the liver and kidney (Fig. 3). From Figure 2 it is evident that

samples. This represents a real increase in concentration with time, and the curve obtained when related to the curve of I^{131}

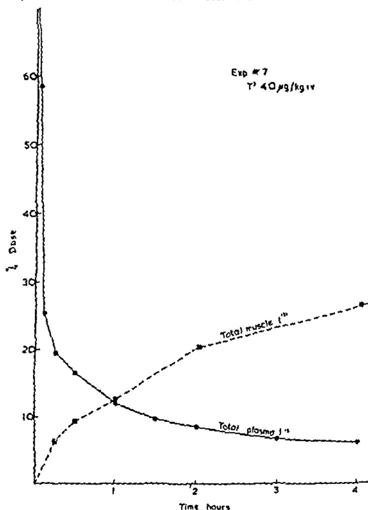


FIG. 1. Curves showing the distribution with time of radioactivity in the plasma and the muscle compartments of a rabbit injected [illegible] with ^{131}I-labelled triiodothyronine. The plasma [illegible]

[illegible] curve.

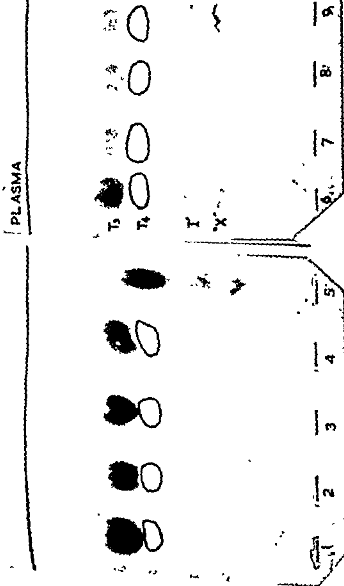


Fig. 2 Chromatograms of plasma in butanol-dioxan ammonia (BDA) at various times after the injection of ^{131}I-labelled triiodothyronine (T_3). Aliquots of plasma together with non radioactive T_3, thyroxine (T_4) and sodium iodide (I^-) were placed on the paper. The samples 1 to 10 correspond to the following sampling times 2, 6, 15, 30, 60, 90, 120, 180, 240 minutes respectively.

[*facing page* 184

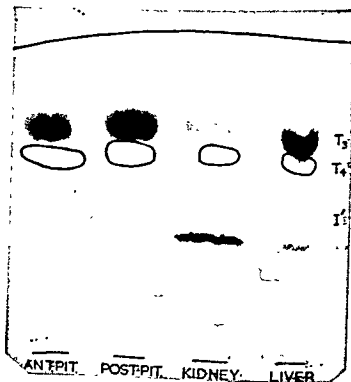


FIG.
and
injec
under Figure 2

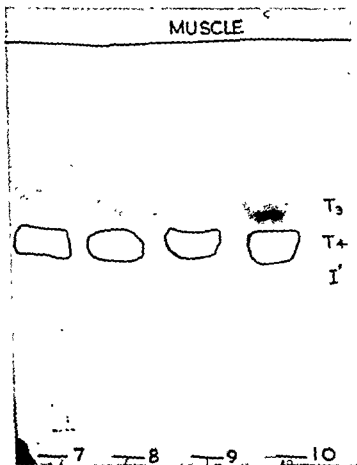
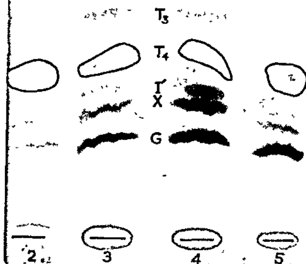


Fig 5 Chromatograms in BDA of butanol extracts of muscle at various times after T_4 injection The sample numbers correspond to the same times given in Figure 2 where the abbreviations are also defined


BILE
T3
T4
T′
X
G
2
3
4
5

concentration in the muscle (Fig. 4) indicates that plasma "x" could be the precursor of the muscle radioactivity.

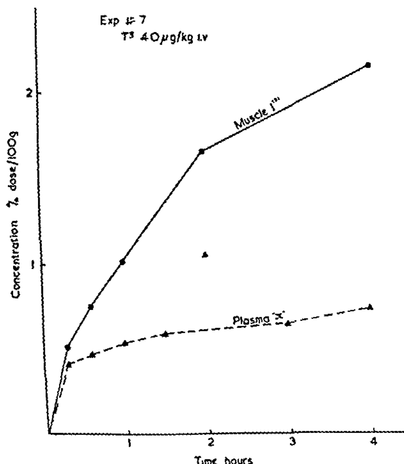


FIG. 4. Curves showing the relationship between the entry of radioactivity into muscle and the appearance of an unknown substance "x" (See Fig. 2) in the circulation, in the same experiment as that shown in Fig. 1.

However, on analysing the muscle it was found that the radioiodine was predominantly in the form of triiodothyronine at all time intervals (Fig. 5); it therefore follows that its

circulating precursor must have contained triiodothyronine. Accordingly attempts were made to isolate and characterize the "x" substance.

Since the liver was probably the major source of plasma "x", it was thought that the bile might provide a suitable concentrate for its isolation. Chromatograms of the bile (Fig. 6) did show several substances in the same region as the plasma compound. Two of these were isolated. The substance labelled G was found to be the glucuronide of triiodothyronine, since treatment with mammalian glucuronidase resulted in the

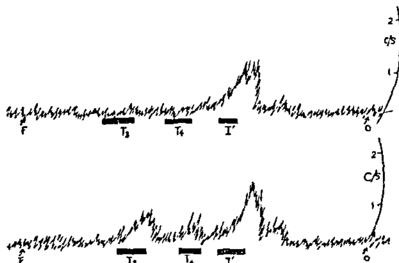


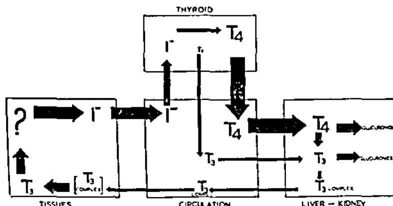
Fig. 7. The effect of acid hydrolysis on an isolated sample of the substance labelled "x" in Figure 6. The tracings give the distribution of radioactivity in the chromatograms (BDA) when scanned under a chromatogram scanner.

before treatment

has been reduced.

formation of triiodothyronine, and this formation was inhibited in the presence of saccharolactone (i.e. a glucuronidase inhibitor). This is presumably the same product first demonstrated by Roche, Michel and Tata (1954) though it is resistant

to hydrolysis with 0·2 N-HCl under the conditions described by these authors. The substance labelled "x", after treatment with 0·2 N-HCl for 2 hours at 54° C, gave rise to triiodothyronine (Fig. 7). In attempts to isolate the "x" substance from plasma, the fractions always contained triiodothyronine as a contaminant together with some iodide. Treatment of this mixture with 0·2 N-HCl resulted in a disappearance of the "x" component and an apparent increase in the triiodothyronine component. This would suggest that the "x" substances



in bile and plasma may be similar, and would support the view that plasma "x" is a triiodothyronine-containing substance Definite proof of this assumption must await the isolation of the "x" substance of plasma under conditions which do not cause its breakdown. Such experiments are in progress.

To unify the results of these and other experiments, the following tentative scheme of thyroid hormone metabolism is presented (Fig. 8). The principal secretion of the thyroid gland is thyroxine, and is accompanied by small and variable amounts of triiodothyronine. These two free amino acids are

cleared from the circulation by the liver and to a lesser extent by the kidney. In the kidney, at least, there is accumulating evidence that thyroxine can be deiodinated to triiodothyronine. A second metabolic pathway for both substances is glucuronide formation, and in the case of triiodothyronine there may be formation of a second complex.

The triiodothyronine formed in the kidney does not return to the circulation (Glitzer, Symchowicz and Gross, 1956); instead, an iodinated substance with a chromatographic mobility similar to that of the triiodothyronine complex appears in the plasma. The kidney and liver may therefore form triiodothyronine from thyroxine, and convert the former into a complex which is passed into the circulation. This transport form of the thyroid hormone would then be taken up by the target tissues where it would be rapidly disassociated to free triiodothyronine. In the tissues further metabolism occurs to give iodide. However, the possibility of the formation of other rapidly metabolized intermediates (represented by the question mark) cannot be eliminated.

Summary

(1) Experiments on the metabolism of small doses of ^{131}I-labelled triiodothyronine in the rabbit are presented.

(2) An iodinated product of triiodothyronine has been demonstrated in the circulation which may be the precursor of the radioactivity found in the extra-hepatic and extra-renal tissues. The liver and kidney contain, in addition to triiodothyronine and iodide, substances similar to the circulating metabolite. This is also true of the bile. In the other tissues, as exemplified by the muscle and the pituitary, the [illegible] hyronine. [illegible] of further [illegible]

(3) There is some evidence to indicate that an unknown metabolite found in plasma and bile is a triiodothyronine complex readily disassociated by dilute acid.

(4) The hypothesis is presented that the major secretion product of the thyroid, thyroxine, is metabolized in the kidney and liver to triiodothyronine which in turn is converted into a complex. This complex is secreted into the circulation and is the precursor of the triiodothyronine found in the tissues.

Acknowledgements

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The assistance of Mr. and Mrs. Alan Brownstone in the preparation of the figures and Mr. C Sutton in their photography is gratefully acknowledged.

REFERENCES

GLITZER, M. S., SYMCHOWICZ, S , and GROSS, J (1956) *Fed. Proc.*, **15**, 76.

GROSS, J. (1954) *Brit. med Bull*, **10**, 218.

GROSS, J. (1955). Brookhaven Symposia in Biology, No. 7, p. 102 New York: Upton.

ROCHE, J , MICHEL, R , and TATA, J. (1954) *C.R. Soc Biol*, *Paris*, **148**, 642.

[Discussion of this paper was postponed until after the paper by Prof. Maclagan—Ed.]

THE DEIODINATION OF THYROID HORMONES *IN VITRO*

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THE discovery of triiodothyronine and of its high metabolic activity, as compared with that of thyroxine (Gross and Pitt-Rivers, 1952, 1953*a*, *b*; Roche, Lissitzky and Michel, 1952) has concentrated attention upon the biosynthesis of the former. A possible route of synthesis would involve secretion of *thyroxine by the thyroid gland, followed by peripheral* deiodination (Gross and Pitt-Rivers, 1953*b*). Although there is abundant evidence for the deiodination of thyroxine in intact animals, the difficulty of demonstrating this process in isolated organ preparations has been an obstacle to the acceptance of this suggested mode of synthesis (Roche and Michel, 1955). Recently, however, the deiodination of thyroxine *in vitro* has been reported by three groups of workers (Albright, Larson and Tust, 1954; Larson, Tomita and Albright, 1955; Maclagan and Sprott, 1954, Sprott and Maclagan, 1955; Cruchaud, Vannotti, Mahaim and Deckelmann, 1955), and the purpose of the present paper is to present some further results on this subject from our laboratory. Although we have studied deiodination of thyroxine in many organ preparations, both by radioactive and non-radioactive methods, the work to be described here was carried out mainly with cell-poor liver extracts obtained as indicated below.

Preparation of Extract

Fresh rat liver is minced into ice-cold 1·15 % (w/v) KCl solution (10 ml./g.). After 5 minutes shaking, the mixture is filtered through gauze and the filtrate diluted tenfold. To

5 ml. of filtrate ($\equiv$ 0·05 g. of liver) are added 5 ml. phosphate buffer at pH 6, and approximately 1 μg. of ^{131}I-labelled thyroxine (supplied by the Radiochemical Centre, Amersham, Bucks).

After a suitable incubation period, 4 ml. of diluted plasma are added (to increase protein concentration) followed by 5 ml. 2 % (w/v) phosphotungstic acid, and 5 drops 1 % (w/v) KI as carrier. After filtration, a suitable amount of the filtrate is counted in a G.M. liquid counter (Veall, 1948) and the results

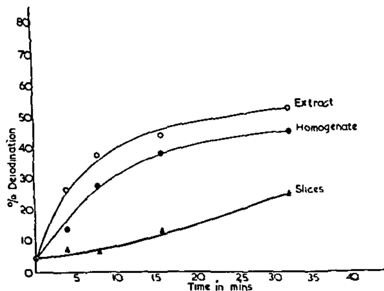


FIG. 1. Rates of deiodination of ^{131}I-labelled L-thyroxine (1 μg) by different liver preparations.

expressed as a percentage of substrate radioactivity. Since the thyroxine is labelled only in the 3′ : 5′ positions, this figure represents percentage deiodination of 3′ : 5′ iodine.

Some typical results are shown in Figure 1, from which it will be seen that the activity of the extract is significantly higher than that displayed by liver slices or homogenates.

General Properties of the Extract

Contrary to our previous experience with homogenates, which proved to be relatively heat resistant, the activity of extracts is readily destroyed by heat. Inactivation is complete after 10 minutes at 100° C, and appreciable destruction occurs at 37° in one hour.

Other properties are very similar to those of homogenates. It will be seen from Figure 2 that the optimum reaction is at pH 6 and the presence of phosphate is essential for maximum activity. The system is aerobic, being completely inhibited by effective exclusion of air. The optimum temperature is around

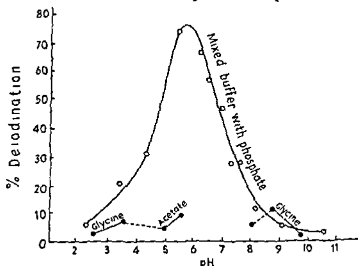


FIG. 2. Effect of pH and buffer composition on rate of deiodination of ^{131}I-labelled L-thyroxine.

37° C, but slow reaction also occurs at temperatures as low as 8° C. At 37° deiodination proceeds almost to completion in one hour. Although there is some variation with different preparations, the average percentage deiodination of between 60–70 per cent indicates that more than one of the 3′ : 5′ iodine atoms are removed. *We have not, however, achieved* 100 per cent deiodination even by the addition of further extract at the end of the reaction.

Effect of Inhibitors

The effect of some inhibitors on the reaction is shown in Table I. These are very similar to those previously reported for the homogenate, and include inhibition by citrate and

Table I

INHIBITORY EFFECT OF VARIOUS SUBSTANCES ON ^{131}I-LABELLED THYROXINE DEIODINATION BY RAT LIVER EXTRACT (0·05 g LIVER)

Inhibitor	*% Inhibition of Deiodination*
Anaerobiosis	100
10^{-1} M-NaCN	100 (10^{-3} M only 25 %)
0·05 M sodium citrate	100
0·05 M sodium citrate + 0·01 M-$FeCl_3$	0
10^{-4} M sodium arsenite	0
10^{-3} M BHDB	59
0·1 M-KI	30
10^{-3} M thiouracil	0
0·5 × 10^{-4} M triiodothyronine	100
10^{-6} M triiodothyronine	30
0·02 % phosphotungstic acid	100

reversal of the inhibition by small amounts of ferric iron. The iron effect appeared reasonably specific as it was not shown by nickel and cobalt (Table II). The inhibitory effect of *n*-butyl 4-hydroxy-3 . 5-diiodobenzoate (BHDB) was of importance

Table II

COMPARISON OF $FeCl_3$, $Co(NO_3)_2$ AND $MnCl_2$ IN THEIR EFFECT ON CITRATE INHIBITION

^{131}I-L-thyroxine and liver homogenate in acetate buffer.
2 hours incubation.

pH	*0·05 M citrate*	*Concn. of salt*	*% Deiodination*
6·0	–	–	67
6·7	+	–	2
6·4	+	0·001 M-$FeCl_3$	71
6·7	+	0·001 M-$Co(NO_3)_2$	2
6·7	+	0·001 M-$MnCl_2$	2

to us in relation to the mode of action of this substance as an antithyroxine agent, and confirms our hypothesis that these effects are produced by inhibition of the deiodination of thyroxine (Wilkinson, Sprott, Bowden and Maclagan, 1954; Maclagan, Sprott and Wilkinson, 1952). Triiodothyronine inhibits at a much lower concentration than that effective in the case of other inhibitors, indicating some degree of specificity. Inhibition with iodide only occurs at high concentration (0·1 M).

Substrate Specificity

The effect of the system on four substrates is shown in Table III. While triiodothyronine (T_3) is deiodinated at about the same rate as thyroxine (T_4), tri- and tetraiodothyroacetic

Table III

DEIODINATION OF DIFFERENT SUBSTRATES BY RAT LIVER PREPARATIONS. (2 HOURS INCUBATION)

Liver Preparation	*% Deiodination*			
	T_4 (5 µg.)	T_3 (6 µg.)	TETRAC (4 µg.)	TRIAC (7 µg.)
Extract (0·05 g.)	66·5, 71·7	—	28·7, 20·2	17·5, 19·8
Homogenate (0·05 g.)	58·7, 54·6	—	—	23·9, 23·9
„ (0·5 g.)	74	58	—	—

acid (TRIAC and TETRAC respectively) (Pitt-Rivers, 1953) were much less readily attacked. Deiodination of the iodoacetic acids was inhibited by the same substances (including BHDB) which inhibited in the case of the iodothyronines.

This difference in the rate of attack with iodothyronines as compared with iodothyroacetic acids is of some interest, particularly as it cannot be explained on solubility grounds. It could be interpreted teleologically either as an indication that the liver was "sparing" the latter compounds, or alternatively that it was unused to dealing with them.

Preparations from other Organs

A wide distribution of the enzyme throughout the body has been previously reported (Sprott and Maclagan, 1955).

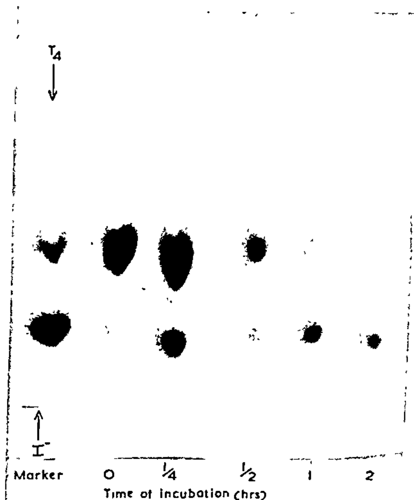


Fig 3. Chromatogram of butanol extracts from ^{131}I-labelled L-thyroxine at intervals after incubation with liver homogenate. Chromatogram run in butanol-dioxan-ammonia system and developed by autoradiography

[facing page 105

Although most active in the liver, activity was also shown by homogenates from kidney, spleen, brain, muscle and adrenal. Only traces of activity were, however, present in thyroid and intestinal mucosa. We have obtained very similar results with the technique of extract preparation described above. No significant activity was found in blood or in bile, and a number of autoxidizable systems composed of haematin, lecithin, cytochrome *c*, ascorbic acid, and $FeCl_3$ also gave negative results.

Chemistry of the Reaction

The fact that the filtrate radioactivity in our experiments is due entirely to iodide has been confirmed by the following technique. The filtrates were treated with a cold dilute acid solution of potassium dichromate to liberate iodine, which was extracted by chloroform. The chloroform solution was re-extracted with aqueous sodium thiosulphate, and the resulting solution investigated by paper chromatography in the butanol-dioxan-ammonia system followed by autoradiography. In this way, it was shown that all the radioactivity was associated with inorganic iodide.

Attempts were also made to identify the organic products of the deiodination by extracting the mixture, after incubation, with acid butanol, followed by chromatography in the butanol-dioxan-ammonia system. The chromatographs were finally developed by autoradiography. The results of a typical experiment are shown in Figure 3 These experiments demonstrated satisfactorily the disappearance of thyroxine and the production of inorganic iodide, and there is also an indication of the accumulation of small amounts of triiodothyronine, particularly after one hour's incubation As noted above, triiodothyronine is itself attacked by the system, which on doubt explains the difficulty of demonstrating its appearance. Attempts have been made to improve on these experiments by the addition of a partially inhibitory concentration of carrier triiodothyronine, with the idea of stopping the reaction at the triiodothyronine stage, but the results were very similar to those shown in Figure 3.

The exact mechanism of the reaction is obscure, the aerobic nature of the process being difficult to reconcile with, for example, the conversion of thyroxine to triiodothyronine, which is one of reduction. It is, therefore, important to stress that the evidence for the existence of triiodothyronine as an end product, both in our work and in that of others, is entirely chromatographic. After Prof. Lissitzky's paper this morning, it is evident that the compound, 3′-hydroxy-3:5:5′-triiodothyronine, should be seriously considered as a possible product of the oxidative deiodination of thyroxine.

Physicochemical Data

Table IV shows the results of attempts to define the nature of the enzyme more closely. It will be seen that activity is unaffected by dialysis, while it was precipitated by one-third

Table IV

EFFECT OF DIALYSIS ON THE DEIODINATION OF ^{131}I-LABELLED THYROXINE (1 µg.) BY RAT LIVER EXTRACT (0 05 g)

State of extract	*Deiodination, % dose*
Undialysed	35 5, 32 2
Dialysed (running water)	39 8, 38 3
Dialysed (static)	34 8, 35 2
Heated control	3 2, 4 4

EFFECT OF PROTEIN PRECIPITANTS

Reagent	*% Activity Precipitated*	*% Activity in Supernatant*
1/3 Saturated $(NH_4)_2SO_4$	52	0
1/2 Saturated $(NH_4)_2SO_4$	52	0
Acetone—20° C	0	0

saturation with ammonium sulphate. This would suggest a soluble protein of moderate molecular weight. The results of [illegible] with an M.S.E. medium [illegible] t 4° C according to the scheme of [illegible] ere consistent with this hypothesis. It will be seen from Table V that no activity was

Table V

EFFECT OF HIGH SPEED CENTRIFUGATION IN 0·25 M SUCROSE AT 4° C ON THE ACTIVITY OF LIVER EXTRACT (0·05 G)

Centrifugal field g min.	*Fraction brought down*	*% Activity in S F.*	*% Activity in Deposit*
10,000	Cell debris and nuclei	100	—
33,000	Heavy mitochondria	91	12
250,000	Light mitochondria	74	14
3,000,000	Microsomes	32	38

carried down with the mitochondria, although a significant proportion was found in the microsome fraction. Further attempts at purification were made by precipitation with acetone at low temperatures, but no active preparations were obtained in this way.

Effects of Thyroid Status

Larson, Tomita and Albright (1955) reported the inhibition of the conversion of thyroxine to triiodothyronine in kidney slices, as a result of thyroidectomy. Experiments carried out in this department by A. J. Feetham have given similar

Table VI

EFFECT OF THYROID FEEDING (2 % THYROIDEUM B P. FOR 18–20 DAYS) ON DEIODINATION OF ^{131}I-LABELLED THYROXINE BY RAT LIVER AND KIDNEY HOMOGENATES (FEETHAM, A J, UNPUBLISHED)

	Liver % Deiodination		*Kidney % Deiodination*	
Control group (6 rats)	52·7	= 40·6 Av	27·6	= 25·5 Av.
	54·4		27·8	
	50·8		28·4	
	28·0		19·9	
	25·2		18·8	
	32·4		30·4	
Thyroid fed group (6 rats)	24·8	= 19·0 Av.	16·9	= 12·3 Av.
	14·2		15·2	
	14·8		18·0	
	20·3		8·4	
	21·3		8·4	
	18·3		6·7	

results (Fig. 4). The degree of inhibition produced by thyroidectomy was, however, unimpressive.

Contrary to the experience of Larson and co-workers, Mr. Feetham was, however, unable to produce any evidence of increased activity by thyroid feeding. It will be seen in Table VI that the results in thyroid-fed animals were in fact *below* those of the control group, *both with liver and with kidney*

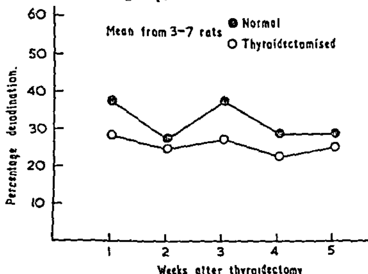


FIG 4. Effect of thyroidectomy on deiodination of ^{131}I-labelled L-thyroxine by liver homogenates (Feetham, A. J, unpublished).

homogenates. It is difficult to explain this apparent discrepancy which may depend on the employment of different dosages or substrate levels. It should also be pointed out that Feetham's experiments were carried out on homogenates, and those of Larson and collaborators on slices. There is the further difference that our measurements were entirely concerned with iodide production, which would not necessarily run parallel with triiodothyronine production.

Conclusion

The existence of an aerobic labile deiodinating enzyme system in the liver and other organs, capable of attacking

thyroid hormones, is of significance in relation to their possible deiodination in peripheral tissues, and suggests that this may well be a normal event in the animal body. The mechanism of the reaction is not established and needs further study. The diminished activity of this system produced by thyroidectomy and by thyroid feeding is also of interest, and suggests that a hormonal control of deiodination may exist and may be of importance as a regulating mechanism.

Acknowledgements

The work has been supported by generous grants from the Medical Research Council, and from the Governors' Discretionary Fund of Westminster Hospital. We are also much indebted to Glaxo Laboratories Ltd, and to Messrs. Smith, Kline and French for gifts of thyroxine and triiodothyronine, and to Dr. J. H Wilkinson for the samples of TRIAC and TETRAC prepared as described elsewhere (Wilkinson, 1956). The Brown Rotary Warburg Apparatus used in this work was obtained through a grant from the Central Research Fund of the University of London

REFERENCES

ALBRIGHT, E. C., LARSON, F. C., and TUST, R. H (1954). *Proc. Soc. exp. Biol., N.Y.*, 86, 137.

CRUCHAUD, S., VANNOTTI, A, MAHAIM, C, and DECKELMANN, J. (1955). *Lancet*, 2, 906.

DUVE, C. DE, PRESSMAN, B C, GIANETTO, R, WATTIAUX, R., and APPLEMANS, F (1955) *Biochem. J.*, 60, 604.

GROSS, J, and PITT-RIVERS, R. (1952). *Lancet*, 1, 593

GROSS, J., and PITT-RIVERS, R (1953*a*). *Biochem J*, 53, 645.

GROSS, J., and PITT-RIVERS, R. (1953*b*). *Biochem. J.*, 53, 652.

LARSON, F. C., TOMITA, K., and ALBRIGHT, E. C. (1955). *Endocrinology*, 57, 338

MACLAGAN, N F, and SPROTT, W. E. (1954) *Lancet*, 2, 368.

MACLAGAN, N. F., SPROTT, W E, and WILKINSON, J. H. (1952). *Lancet*, 2, 915.

PITT-RIVERS, R (1953) *Lancet*, 2, 234.

ROCHE, J, LISSITZKY, S, and MICHEL, R. (1952). *C.R. Acad. Sci., Paris*, 234, 997.

ROCHE, J., and MICHEL, R. (1955) *Physiol. Rev*, 35, 583.

SPROTT, W E., and MACLAGAN, N. F. (1955). *Biochem J.*, 59, 288.

VEALL, N (1948). *Brit. J. Radiol. N.S*, 21, 347.

WILKINSON, J. H. (1956) *Biochem J*, 63, 601.

WILKINSON, J. H., SPROTT, W. E, BOWDEN, C. H, and MACLAGAN, N. F. (1954). *Biochem. J.*, 56, 215.

DISCUSSION

Lardy. I should like to make a few comments about the whole homogenate versus the extract, and reasons for the heat stability. When these data were first published by Prof. Maclagan they looked so much different from the Larson-Albright system that we did a few com-
... The purpose of the experiments was to see whether or not the
... caused
... ds

enzymic catalysis.

Now, concerning the reasons for the differences between your experiments and those of Larson and co-workers on the hyperthyroid animal, I feel there may be a difference in the tissue; they used kidney slices,
... kidney too?

... yroid
feeding, of course, but it did seem ...

Taurog: Dr. Lardy has just said something that strikes me as very interesting. You say it is well known that sulphydryl compounds strip iodine from thyroxine?

Lardy: No; from other halogenated compounds. Someone in this country has reported in the *Biochemical Journal* the dehalogenation of alkyl halides by glutathione.

Michel: But in this paper, possibly, the same author states that deiodination occurred in aliphatic halogenated compounds but not in aromatic compounds.

Lardy: According to Larson, glutathione will deiodinate thyroxine.

Taurog: That is certainly very important to know in this chromatography.

Maclagan: Yes, but still 10 minutes' heating would not destroy your glutathione?

Lardy: No.

Maclagan: So that the deiodinating activity of our liver extracts could not be due to glutathione.

Lardy: No, the deiodination of thyroxine by your liver *extracts* is clearly an enzymic process.

Taurog: I just wanted to ask Dr. Gross about the nature of this triiodothyronine complex that he mentions. I did not quite understand what

suggestions as to what this complex might mean I know that Prof. Roche and Dr Michel have thought about a number of complexes.

P.-Rivers. Dr. Michel, you did actually do some work, didn't you, with radioactive sulphate?

Michel: Yes, but without success We first tried injection of radioactive sulphate and also radioactive cysteine. Despite long treatment with

Michel Yes, but compound S was found when the blood is withdrawn 4 or 5 hours after injection of triiodothyronine and 20 or 30 hours after injection of thyroxine. It is possible that if thyroxine is deiodinated

erroneous.

P.-Rivers: Did compound S have the same R_F as your complex?

Gross. I cannot really say because we never used the same solvent.

Michel I think it has the same R_F. In butanol/ammonia, is this compound between the origin and iodide?

Gross: Yes.

Michel: It is surely not the glucuronide conjugate because we have

spleen or from bacteria.

Lissitzky: Prof. Maclagan, you showed an optimum activity in function of pH with your system, and the experiments you presented today were done on the 1 µg. scale. I do not remember if this is the same as the result

because solubilities come in.

Thibault: Prof. Maclagan, do you think that the inhibiton of thyroxine by triiodothyronine might be due to a reversibility, perhaps, of the transformation of thyroxine to triiodothyronine, and that excess of triiodothyronine displaces the equilibrium in favour of thyroxine?

Maclagan: Perhaps, but we were thinking more of the competition for the enzyme sites, where the triiodothyronine would be in fairly gross excess as compared with the labelled thyroxine

Thibault: At what concentration does triiodothyronine inhibit deiodination?

Maclagan: 10^{-5} molar about.

Roche: As far as I remember, we had similar results for the deiodination

a specific activity ranging, usually, between 20 and 50 mc/mg., and they send it to us by air.

Michel: We have triiodothyronine with an activity of 0 5 mc/μg. triiodothyronine and it is quite impossible to inject that.

Gross: You will appreciate that the chromatograms were not done on mice but on rabbits, and the dosage used was 5 μg./kg. (i e. 100—250 μc./kg)

Maclagan · As regards the preparation of the substrate I should like to say how much help we have had from Dr Michel's trick, which he told me last year, of chromatographing the thyroxine before one uses it and then simply cutting off the strip of paper, keeping it dry and eluting as much paper as is required for each experiment. That has been a great [illegible]

and we cut 2 or 3 cm of paper when we need it, and after elution with butanol saturated with water, we concentrate and then use the com- [illegible]

[illegible] ¹⁴C-labelled organic compounds (such as choline or glycine) are capable of extensive decomposition under the influence of their own radiation, and that the decomposition was decreased by mixing the crude product in powder with some inert material, like talc or by diluting it when in solution.

[illegible]

Taurog. Yes, that certainly is true

Vannotti · I also often observed the decomposition of the chromatographically-pure labelled thyroxine in propylene glycol solution. The best way to prevent this decomposition is the lyophilization of the thyroxine

[illegible]

other solvents.

GENERAL DISCUSSION

P.-Rivers. Before we start this discussion I want to draw your attention to the fact—though I am sure it is quite unnecessary—that almost each one of us has individual names, a lot of them "pet" names, for the different thyroid-like compounds. I think it is time there was some sort of uniformity about this.

One of the troubles has arisen because thyroxine and triiodothyronine have been called T_4 and T_3 for a very long time—it is an easy thing to write on your chromatograms—and Prof. Querido has extrapolated backwards to diiodotyrosine and monoiodotyrosine as T_2 and T_1. Now, this is quite unsystematic: T at least should refer to the same nucleus, namely thyronine. In view of Dr. Michel's and Prof. Roche's discoveries: *mono-* and *diiodo-*thyronine, I think that Prof. Querido should withdraw his abbreviation, and that T_1, T_2, T_3 and T_4 should refer to iodinated thyronines. It was perfectly clear to me on the marked chromatograms this morning that what Dr. Michel called $3:3'$-T_2 was $3:3'$-diiodothyronine. If we could in future keep these abbreviations for the thyronines, I think it would lead to much less confusion.

If we accept this, then we have to produce abbreviations for *mono-* and diiodotyrosine, and as most of the abbreviations really come from people writing on their chromatograms and on their autoradiographs they should be fairly short. The old MIT and DIT seem to me quite unequivocal; you may not care for them very much, but at least you should know from long usage that MIT and DIT refer to monoiodotyrosine and diiodotyrosine. With regard to the acetic acid derivatives—again for the sake of brevity—I started shortening them to TETRAC and TRIAC *in* ... notebooks and on my chromatograms, and a lot of ... They are not per- ... and they do

down as ... n

the literature! I do not know quite what to do about these compounds and would welcome any suggestions.

Gross· I wonder if we could have the di- and triiodothyronines finished first because I am in doubt in my own mind about the conventions for the 3 : 3' : 5'-tri and the 3 : 5 : 3'-tri, and the 3 : 3'-di and the 3 : 5-di, etc.

P.-Rivers: There is no ambiguity about this: 3 : 3' : 5'-T_3 for 3 : 3' : 5'-triiodothyronine, and 3 : 3'-T_2 for 3 . 3':-diiodothyronine, is there? 3-refers to one ring, 3' to the other.

Roche. Then you will accept the numbers?

P.-Rivers: Oh yes, surely, they made Prof. Michel's chromatograms extremely clear.

Lissitzky. Would it not be more homogeneous to call the acetic derivatives T_3AC and T_4AC in abbreviations?

Roche· Yes, if we accept T

Thibault. But it is difficult to say.

Barker. I should like to object to this T_2, T_3, T_4, T_3AC, T_4AC and so on, because at various larger meetings we have people using them so indiscriminately T_2 is so easy to say that it is applied to all sorts of compounds, for instance, it has been used for diiodotyrosine. I think that TRIAC and TETRAC are understandable, even if it is going to end up with TRIPE or TETROPR for the propionic acids.

Again we put in numbers as prefixes, for example, 3 3' · 5'-TRIAC.

Lardy It is not consistent to write T_3AC because T really means "thyronine" and in that case it is not thyronine.

P.-Rivers There is another reason for calling the acetic acid analogues TRIAC and TETRAC and that is that they are now being supplied in reasonable quantities for research purposes under those names, and if any of you want them, all you do is write to Glaxo Laboratories and you will get them under those names. Unless there is a very strong objection to them they seem to me fairly unambiguous

With regard to the thyronines, I was not proposing that when I read my next paper I should say T_2, T_3, T_4 but merely that when

writing four lines for each name; they really are very clumsy and I

do not think there is much to recommend them. TETRAC is surely better than tetraiodothyroacetic acid.

Barker: And since the diiodo forms are appearing, could we not use DIAC?

P.-Rivers: I do.

Wilkinson: While this is being discussed, would it not be as well to specify the names TRIAC and TETRAC for those particular isomers; and if other isomers are used or referred to, then the positions should be noted.

P.-Rivers: Yes.

Fraser: There is only one small aspect of this that I think might lead to confusion and that is the MIT and the DIT.

Fraser: That is what I thought, yes.

Gross: I wonder now why we cannot make this Ty_1 and Ty_2? ... which becomes ... uch better than ... tyrosine?

abbreviations.

P.-Rivers: TYR? Yes, they probably have.

Taurog: Well, there has to be some continuity with previous reports, so why not leave it MIT and DIT; I don't think you want a sudden change from what you have been using.

P.-Rivers: If you wrote MIT in capital letters I do not think anybody would say that they do not know what you mean by it.

Taurog: Yes, I agree.

Michel: What about Tx for thyroxine?

Barker: And unfortunately, in the American literature Tx has come to mean thyroidectomy and Hx hypophysectomy! I think ... t T_4 and T_3, aren't we? ... r suggestions? ... become physiologically significant we can try again!

P.-Rivers: Then we will get together again!

I didn't want to be dictatorial about this but just to suggest that we should understand each other better, especially where there is any language difficulty, if we did try and use the same abbreviations

Barker: I have some slides which have a bearing on some of the work mentioned this morning about *in vitro* effects of thyroxine

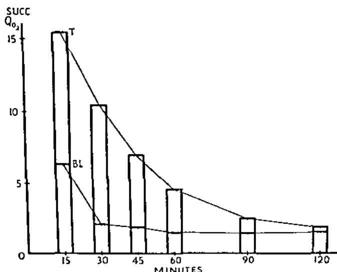


FIG. 1 (Barker) Effect of added thyroxine on "succinoxidase" activity of rat heart homogenate

hour and then 90 and 120 minutes, the rapid falling-off of this system and the tremendous effect that one obtains with the addition of thyroxine. I think you can see very easily the difficulty in talking about stimulation when the things are falling off as much as this.

In corroboration of the Clarke-Ball work mentioned this morning by Dr. Lardy, in Figure 2 I have shown the marked inhibition which one can get in the malic dehydrogenase system with the addition of thyroxine. I should say that this is not a true malic

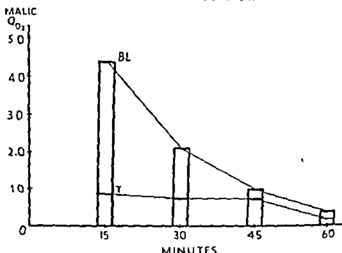


FIG. 2 (Barker) Effect of added thyroxine on malic dehydrogenase activity of rat heart homogenate.

Table I (Barker)

COMPARISON OF THE EFFECT OF VARIOUS THYROXINE ANALOGUES ON SUCCINOXIDASE AND MALIC DEHYDROGENASE ACTIVITIES OF RAT HEART HOMOGENATE

COMPOUND	% of T_4 effect		COMPOUND	% of T_4 effect	
	succ.	malic		succ	malic
HO–(ring; I, I)–O–(ring; I, I)–CH_2 CH(NH_2) COOH	100	100	HO–(ring; I, I)–C(–ring–COOH)=(ring; I, I)=O	95	90
HO–(ring; I)–O–(ring; I, I)–CH_2 CH(NH_2) COOH	82	90			
HO–(ring; I)–O–(ring; I, I)–CH_2 CH_2 COOH	96	94	HO–(ring; I, I)–CH_2–CH COOH, NH–C=O–(ring; I, I)–OH	94	94
HO–(ring; I, I)–O–(ring; I, I)–CH_2 COOH	94	97	HO–(ring; Br, Br)–O–(ring; Br, Br)–CH_2 CH(NH_2)COOH	61	67
HO–(ring; I)–O–(ring; I, I)–CH_2 COOH	86	95	HO–(ring; Cl, Cl)–O–(ring; Cl, Cl)–CH_2 CH(NH_2) COOH	8	21

inactive, which Clarke and Ball interpret as meaning that thyroxine and DPN have some sort of an antagonistic effect.

In comparing the effect of various analogues with thyroxine on the "stimulation" of succinoxidase and the depression of the

ularly to tetraiodophenolphthalein, the gall bladder dye, which has absolutely no metabolism-stimulating ability and yet which you will see has as much *in vitro* activity as thyroxine. Also a diiodotyrosine derivative is shown, diiodohydroxybenzoyldiiodotyrosine, which has the advantage that it will not polymerize easily to form thyroxine. That also has no metabolism-stimulating ability and yet it is as effective on both succinoxidase and malic dehydrogenase as is thyroxine.

P.-Rivers. I have another compound which will not polymerize to give thyroxine which you might like to try, Dr. Barker, and that is acetyldiiodotyrosyldiiodotyrosine which just will not give thyroxine under mildly alkaline oxidative conditions, whereas most of the other diiodotyrosine derivatives do.

Lardy: I wonder if we might have opinion expressed, without leading to too much of an argument, as to whether people now agree that thyroxine must be converted to triiodothyronine before it is physiologically active or whether they believe that thyroxine itself is active without being converted to other compounds.

P.-Rivers: I believe all these compounds are probably active. At present there is no evidence against their being active in their own right, as far as I can see.

Gross· I should rather like to stress the physiological viewpoint especially in the face of what appears to be non-specificity when we become less than physiological

In the animal, so far, the main compounds that we see are thyroxine and triiodothyronine. Of these, thyroxine doesn't appear to get into target sites readily and triiodothyronine does,

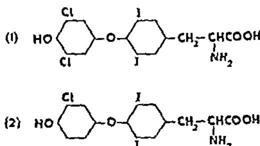
thyroxine.

Lardy: I think that the discussion should be about the intact animal.

P.-Rivers: I was going to talk of the intact animal level. We have been doing some work by the goitre prevention assay method in

rats on the comparison between various tetra- and tri-halogenated thyronines. You have probably seen the preliminary report where tribromothyronine was many times more active than tetrabromothyronine and trichlorothyronine than tetrachlorothyronine. The sulphur analogue of triiodothyronine is enormously more active [illegible]

ing tetra-compound, until we came to this pair of compounds. The side-chain in all cases in this series is alanine.



Now, you have got to remember this: trichlorothyronine is much more active than tetrachlorothyronine but compound (1) is about 5 times as active as compound (2). It is the only pair in a large series of compounds where the tetra-halogenated compound is unequivocally more active than the tri-halogenated compound.

My explanation is that these compounds are active in their own [illegible]

[illegible] some
[illegible] nine;
[illegible] so to
[illegible] way
[illegible] hings
going across [illegible] kely.

Barker: Dr. Gross, if the thyroxine does not get into the target cell, where does the triiodothyronine come from? Does it all come from the liver, and does the thyroxine get into the liver?

Gross: There is no question about that—the thyroxine gets into the liver and into the kidney. When you inject thyroxine you can demonstrate the iodine concentrating primarily in those two organ sites and really not to any extent except in the posterior pituitary which we will leave out of the discussion!

Barker: Well, Dr. Halmi and I have been discussing a point of interpretation which bothers both of us. There is no question of intracellular penetration of thyroxine if the tissue concentration goes above that explainable by the sodium space. We do question the interpretation that there must be no cellular penetration just because the level does not go above that which can be explained by the sodium space. Suppose 10 per cent of the material goes into the cell—you cannot tell that.

Gross: No, the point is very well taken; certainly one cannot say categorically and it would be foolish to maintain that attitude; but on the relative abilities of these two thyroactive substances to penetrate cells in the animal, one of them with a higher manifest biological activity—and, to my knowledge, the *highest* manifest biological activity—does get into the target cells readily.

Querido There are two points that I want to raise. Is the explanation for the better distribution of triiodothyronine in the cells possibly that it is more loosely bound to the plasma proteins [illegible]

[illegible]

[illegible]

—is metabolized just because it is a product of metabolism. Wo[illegible]

In [illegible]

qu[illegible]

bec[illegible]

muscular exercise on the running wheel.

Barker: Wulf at Bethesda has evidence that dinitrophenol increases the peripheral disappearance of thyroxine.

Querido. I wonder if doing experiments along that line—by push[illegible]

run[illegible]

Fr[illegible]

bol[illegible]

increased BMR.

[illegible]

Querido: We recently have proof that muscular activity enhances

disappearance of thyroxine. We started out by repeating the *experiment on the effect of* swimming but the result was negative. The question arose whether the experiment was well planned. The *questions were · what are the smallest quantitative differences* that can be detected with the analytical procedure? *Secondly,* suppose the disappearance rate is 100 per cent quicker, how long must the animal be exposed to this particular situation to detect a difference? On that basis we calculated that if there is a 100 per cent faster disappearance of radiothyroxine from the peripheral blood, the experiments had to last between 6–12 hours in order to see something in the data.

The next experiments were as follows: thyroidectomized rats

rest periods in between, for 12 hours. The results are seen in Table I.

Table I (Querido)

INFLUENCE OF 12 HOURS RUNNING WHEEL ON ^{131}I DISTRIBUTION IN THYROIDECTOMIZED, L-THYROXINE-MAINTAINED RATS

(24 hours after 5μg. ^{131}I thyroxine)

	Per cent dose ^{131}I		
	6 trained 12 hours on running wheel	*8 controls trained*	$\Delta_{\text{I-II}}$
Serum . .	0 89 ± 0 19	1·19 ± 0 18	p : 0 01–0·001
Carcase (g) .	0 132 ± 0 016	0 157 ± 0 023	0 01–0·01
Liver (total) .	8 01 ± 1 75	10 96 ± 1 74	0 001

Here are just a few data from these results. The first column gives 6 animals trained and running 12 hours, and in the second *column* there are data on 8 trained animals not running. There

…active thyroxine

I think in other

onsideration that

within the margin

And I wonder, in a third experiment we are planning now, after

we have done the influence of cold and of exercise, what the effect of increasing the temperature, of hyperthermia, will be.

Trotter: I was just a bit worried about the consequences of Prof. Querido's line of thought. If we are going to say that hypermetabolism due to muscular exercise increases the utilization of thyroxine, and that hypermetabolism due to dinitrophenol increases the utilization of thyroxine, what about the hypermetabolism produced by thyroxine? It would seem likely that that also would increase the utilization of thyroxine, which would lower blood levels which in turn would stimulate the pituitary and the thyroid. All hyperthyroidism should be self-perpetuating.

Querido My point was whether you would be able to find certain metabolites more readily in one state than in another. This could put us on the track whether we have to consider them as specific hormones or general breakdown products. It was only a suggestion and I wonder whether there could be something in it.

Wilkinson: Table I shows some results of the examination by chromatographic procedures of plasma extracts from patients in various thyroid states. The chromatograms were developed in a variety of different solvents, not all the solvent systems, however, were used in any one case because there was not enough extract to go round. The compounds were detected by means of ceric sulphate-arsenite reagent which is sensitive down to about 0·1 or 0·05 µg. on the chromatogram.

Table I (Wilkinson)

The Detection of Thyroxine and Triiodothyronine in Human Plasma in Various Thyroid States

Compounds detected	*Number of cases*			
	Thyrotoxic	*Euthyroid*	*Hypothyroid*	*Myxoedema*
Both	15	9	0	0
Triiodothyronine alone	0	1	0	0
Thyroxine alone	0	2	4	2
Neither	0	0	1	8

The identity of triiodothyronine depends on its having the correct R_F value in a number of different solvent systems. We have not been able to isolate sufficient for chemical analysis or

anything like that, so it is conceivable that we may be wrong, but I think that is unlikely.

We detected both compounds in all the thyrotoxic cases, and in

to

me

patient?

Wilkinson: In one patient only. Whether that means anything, I do not really know.

Querido: Did he have a low PBI?

Wilkinson: We have not done PBI's.

Taurog: Is it possible that your failure to find both thyroxine and triiodothyronine in the euthyroid individuals merely reflects a lower PBI in those individuals and, therefore, a limit to the sensitivity of your method?

Wilkinson: I think that is probably the case.

Taurog: And say a thyrotoxic might have twice as much PBI as the euthyroid, and if the euthyroid had the same percentage of triiodothyronine, it would be a lower amount for you to detect, simply because the total amount is less.

Wilkinson: Yes, quite true, but, as you see, both compounds were found in most of the euthyroids.

Taurog: Well, I thought that you were going to suggest that

HEPATIC REGULATION OF THYROXINE METABOLISM

A. VANNOTTI

Clinique Médicale Universitaire, Lausanne

As early as 1925, Abelin and his co-workers observed the high concentration of thyroxine in the liver. This was confirmed by Krayer in 1928 and later by other authors. Gross and Leblond (1947) demonstrated that two hours after the injection of labelled thyroxine into the rat, 9–24 per cent of the hormone is found in the liver. Lipner, Klitgaard and Barker (1952), with the same method, have shown that the thyroxine enters the hepatic cell, and is distributed as follows: 18 per cent in the nuclei, 23 per cent in the mitochondria and 54 per cent remains in the supernatant fraction.

The biliary excretion of thyroxine was described in 1919 by Kendall. Gross and Leblond (1947), using ^{131}I-labelled thyroxine, confirmed the presence of the hormone in the bile. Taurog, Briggs and Chaikoff (1952) have observed that in addition to thyroxine, the bile contains glucuronic acid compounds of thyroxine. Roche *et al.* (1954) also found triiodothyronine and its glucuronic acid compound in the bile. Albert and Keating (1952) have demonstrated the existence of an enterohepatic circulation of the thyroid hormone. Recently Roche, Michel and Tata (1953) showed that after ligature of the bile duct in the rat, glucuro-compounds of thyroxine and of triiodothyronine pass into the circulating blood.

Following these observations we studied clinically and systematically the metabolism of iodine and organic iodine compounds by measuring the ^{131}I fixation in the thyroid, the PBI level in blood and the conversion index, as well as chromatography of butanol extracts of plasma and bile after

administration of ^{131}I to normal people and to patients suffering from obstructive jaundice and infectious hepatitis.

The results of these observations lead to the following conclusions (Scazziga, Béraud and Vannotti, 1955): in normal people, in agreement with the results previously

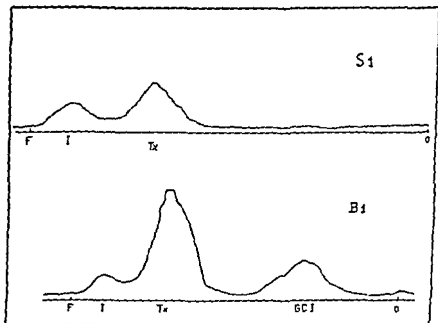


FIG 1.
S1—chromatogram of butanol extract of plasma in normal people.
B1—chromatogram of butanol extract of bile in normal people.
I—iodide.
Tx—thyroxine.
GC I—glucuro-compound I.
O—origin.
F—front.

obtained by many workers, we never found glucuro-compounds in circulating blood, but we found them in the bile, together with thyroxine (Fig. 1).

The glucuronic acid compound of thyroxine, often associated with other known or unknown compounds, appears regularly in the blood plasma in all the cases of obstructive jaundice

(biliary stones or cancerous obstruction of the bile system) in which the hepatic function tests are normal (Fig. 2).

In infectious hepatitis, in which the lesion in the liver cell was confirmed by hepatic tests, we found the glucuronic acid compounds neither in the blood plasma nor in the bile. On the other hand, we regularly found thyroxine in the bile (Fig. 3).

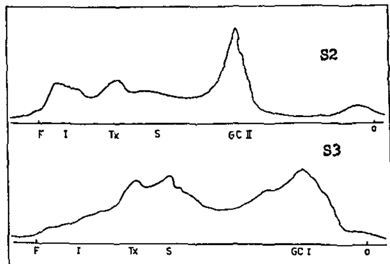


FIG. 2.
S2 and S3—chromatograms of butanol extract of plasma of patients with obstructive jaundice.
I—iodide
Tx—thyroxine.
GC I and GC II—glucuro-compounds I and II after Roche.
S—unknown compound after Roche.

These observations showed, therefore, that in man the normal liver regularly excreted thyroxine and glucuronic acid compounds of this hormone through the bile. By the obstruction of the biliary tract the glucuronic acid compounds pass with the bile into the blood, while in diffuse infectious lesions of the liver the hepatic cell is not able to conjugate the thyroxine with glucuronic acid. We confirmed these clinical observations with some experiments in the rat.

By chromatography of liver extracts of normal rats which had received subcutaneously 5–10 μc of labelled and chromatographically pure thyroxine or triiodothyronine, we found the injected hormone in the liver three to six hours after the injection. At the same time, we also found an important quantity of glucuronic acid compound and the S-compound

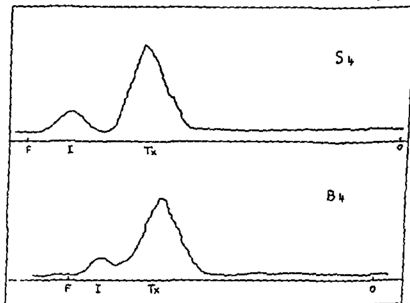


FIG 3.
S4—chromatogram of butanol extract of plasma in patients with infectious hepatitis.
B4—chromatogram of butanol extract of bile in infectious hepatitis
I—iodide.
Tx—thyroxine.

of Roche. On the other hand, we did not see these compounds in the chromatograms of kidney extracts.

We obtained the same results after a slight liver lesion with allyl formate poisoning, but in the severe liver lesion with allyl formate we observed that the glucuro-conjugation of the thyroid hormones (thyroxine as well as triiodothyronine) ceased (Béraud, Scazziga and Vannotti, 1956) (Fig. 4).

The determination of the thyroid function in twenty

patients suffering from infectious hepatitis showed an elevation of the PBI level with a low fixation curve of ^{131}I in the thyroid and a diminution of the values of the conversion index. *All these changes have been statistically verified (Fig. 5).*

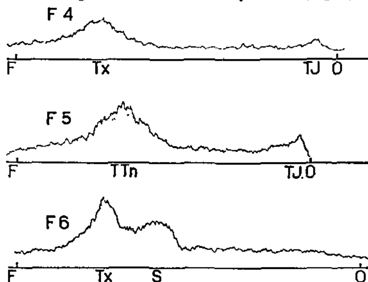


FIG. 4.

In none of these chromatograms did we see the glucuro-compounds of thyroxine and triiodothyronine.
TTn—triiodothyronine.

The elevation of the PBI rate in infectious hepatitis cannot be interpreted as a sign of thyroid hyperfunction because the fixation curve and the conversion index are low. We are obliged to think that the hyperthyroxinaemia in this case is the result of an insufficient destruction of the hormone in the hepatic cell, and possibly an insufficient biliary elimination of thyroxine. The observation of normal quantities of labelled thyroxine in the bile in infectious hepatitis goes against thi

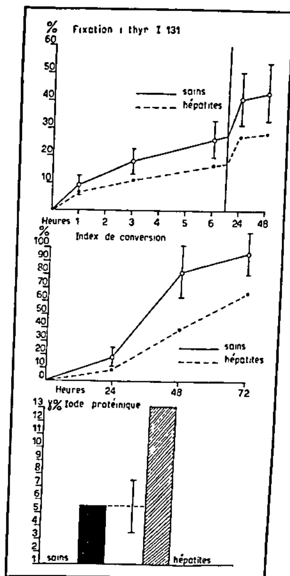


FIG. 5. ¹³¹I-fixation curve in the thyroid in normal people and in patients with infectious hepatitis.
Conversion index in normal people and in patients with infectious hepatitis
PBI values in normal people and in hepatitis patients

last hypothesis, but it is to be noted that the total bile excretion through the intestine in this case is decreased.

The increase of the thyroxine level in the blood provokes an inhibition of the pituitary gland which explains the low fixation rate of ^{131}I in the thyroid and the low conversion index.

Finally, using slices of rat livers, we tried to study the transformation of thyroxine into triiodothyronine and

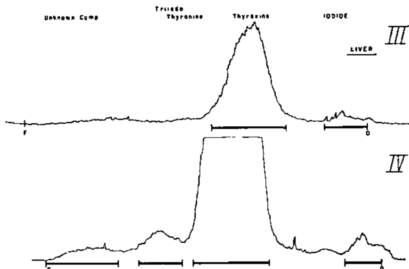


FIG 6. Transformation of thyroxine into triiodothyronine and TETRAC by incubation of liver with labelled chromatographically pure thyroxine

tetraiodothyroacetic acid (TETRAC) with a technique analogous to that of Albright, Larson and Tust (1954) in order to compare the deiodinization and oxidation of thyroxine in the liver and kidney. We arrived at the conclusion that the transformation of thyroxine, which usually appears after 4–7 hours of incubation of kidney or of heart, in the case of liver slices always began after 15–30 minutes (Fig. 6)

In rats previously treated with thyroxine this transformation appears in the liver slices later (1–2 hr.). By experimental liver damage with allyl formate the formation of the triiodothyronine + TETRAC spot was not observed.

All these experiments and clinical observations stress the importance of the liver for the peripheral regulation of the thyroid hormones. In fact, the liver is able to excrete thyroxine through the bile and to convert it into a glucuronic acid compound. This process is a physiological function and represents one of the ways in which the liver eliminates and inactivates the thyroid hormones. On the other hand, through deiodination and oxidation of the thyroxine to triiodothyronine and TETRAC, normal liver can influence the utilization of the thyroid hormone. All these mechanisms can be disturbed by liver damage.

Finally, I wish to mention that in human pathology we have another example of altered thyroxine excretion. This is the case in lipoid nephrosis, in which we (Cruchaud, Mahaim, Scazziga and Vannotti, 1954) found a very high fixation of ^{131}I in the thyroid, with a normal or high conversion index. By means of chromatography of the urine extracts we found that in lipoid nephrosis the urine contains not only inorganic iodine but also protein-bound iodine, and especially thyroxine. In this renal lesion (but not in nephritis) the low plasma protein level explains the low PBI, the thyroxine, following the protein carriers, leaves the body via the kidney.

Under these conditions, the thyroid function is increased, while the patient shows the symptoms of an hypothyreosis, losing the iodine and the thyroxine through the urine.

The clinical observations stress the necessity of studying not only the pituitary and thyroid, but also the peripheral regulation of the thyroid hormones.

REFERENCES

ABELIN, J., and SCHEINFINKEL, N. (1925). *Ergebn Physiol.*, 24, 640.
ALBERT, A., and KEATING, F. R., Jr. (1952). *Endocrinology*, 51, 427.
ALBRIGHT, E. C., LARSON, F. C, and TUST, R. H. (1954). *Proc. Soc. exp. Biol, N.Y.*, 86, 137.
BÉRAUD, TH., SCAZZIGA, B. R., and VANNOTTI, A. (1956). *Acta Endocr., Copenhagen*, 22, 55.
CRUCHAUD, S., MAHAIM, C, SCAZZIGA, B., and VANNOTTI, A. (1954). *Schweiz. med. Wschr.*, 84, 476.
GROSS, J., and LEBLOND, C. P. (1947). *J. biol. Chem.*, 171, 309.

KENDALL, E. C. (1919). *Endocrinology*, **3**, 156.
KRAYER, O. (1928). *Arch. Path. Pharm.*, **128**, 116.
LIPNER, H. J., KLITGAARD, H., and BARKER, S. B. (1952). *Endocrinology*, **51**, 406.
ROCHE, J., MICHEL, O., MICHEL, R., and TATA, J. (1954). *Biochim. biophys. Acta*, **13**, 471.
ROCHE, J., MICHEL, R., and TATA, J. (1953) *Biochim. biophys. Acta*, **11**, 543.
SCAZZIGA, B. R., BARBIERI, L. L., and BÉRAUD, TH. (1955). *Schweiz. med Wschr*, **85**, 471.
SCAZZIGA, B. R., BÉRAUD, TH., and VANNOTTI, A (1955). *Schweiz. med. Wschr.*, **85**, 1019.
TAUROG, A., BRIGGS, F. N., and CHAIKOFF, I. L. (1951) *J. biol Chem.*, **191**, 29.
TAUROG, A, BRIGGS, F. N., and CHAIKOFF, I. L (1952). *J. biol. Chem*, **194**, 655.

DISCUSSION

Roche: Prof. Vannotti, I have some figures on the excretion of the various iodothyronines and of TRIAC by the liver in thyroidectomized rats, just to compare what happens with the various iodothyronines and with thyroxine.

[illegible] which is an exact confirmation

3:5.3′-triiodothyronine and thyroxine, except that with thyroxine there is less of the ketonic derivative—it is easier to find it in the urine.

With the two other compounds the pictures are in some respects

right one is the same product treated by bacterial β-glucuronidase;

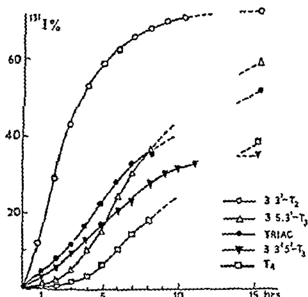


FIG. 3 (Roche). Recovery of ^{131}I in bile of rats after treatment *with different labelled iodothyronines expressed in* per cent of total radioactivity injected.

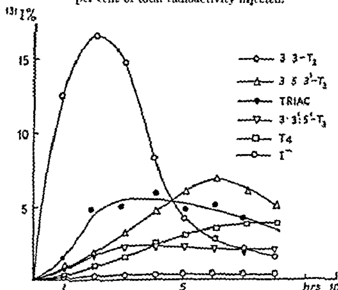


FIG. 4 (Roche). Kinetics of biliary elimination per hour of different iodinated compounds expressed in per cent total radioactivity injected.

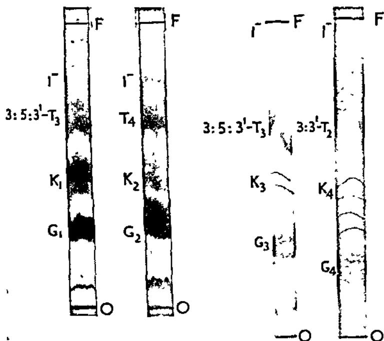


FIG. 1 (Roche). Autoradiograph of a one-dimensional chromatogram of bile collected from rats injected with different iodothyronines (solvent collidine/water/ammonia).
T_2—diiodothyronine, T_3—triiodothyronine, T_4—thyroxine, O—origin, F—front
G_1, G_2, G_3, G_4—spots of glucuronides of the various iodothyronines.
K_1, K_2, K_3, K_4—spots of α-keto acids derived from iodothyronines.
I^-—iodide Other spots—unknown compounds.

[facing page 224

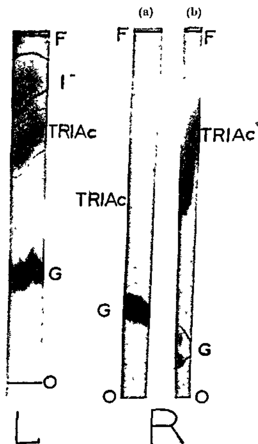


FIG. 2 [illegible] ...ule

(b) hydrolysis of compound G with bacterial β-glucuronidase.
G—TRIAC glucuronide.

[illegible] there are some differences but not very large [illegible]

compared to the others.

Figure 5 is concerned with the urinary excretion of radioactivity after administration of the same doses as before of the same substances. Here, also, you see that the position of the 3:3′-diiodothyronine is exceptional. This could possibly be used to interpret the fate of the 3:3′-diiodothyronine in the organism. We find very frequently this substance as a sort of end-point of deiodination of triiodothyronine.

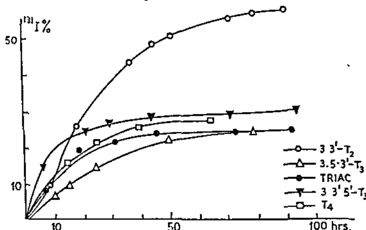


FIG. 5 (Roche) Recovery of ^{131}I in urine of rats after treatment with different labelled iodothyronines expressed in per cent of total radioactivity injected

On the other hand we never found it in the urine. It seems to indicate that really if 3:3′-diiodothyronine is formed as a sort of end-point of the deiodination process, it may be that it is also the substance which is destroyed by the breaking of the phenoxy bridge between the two benzene rings.

Pochin: I should like to make a point about the speed of entry of thyroxine into the liver in the human where it appears that if one gives intravenous radioactive thyroxine, and follows the plasma and the [illegible]

[illegible] and so presumably leaving the liver per minute. That is interesting in relation to figures that Myant has obtained on human

roxine in man and it does appear that the equilibrium figure, the liver space, is about equal for triiodothyronine and for thyroxine. They both enter a thyroxine or triiodothyronine space of something over seven litres. And they enter it very

circulating thyroxine, and in

triiodothyronine, is in the live

cannot be accounted for in the plasma must be in the liver, at least during these early stages.

I think the question arises as to whether the amount of the enterohepatic circulation, and so the control of circulating thyroxine in man, is not very different in degree from what it is obviously shown to be in the rat where there is very substantial flow of thyroxine into the bile, with subsequent intestinal reabsorption. In man, if these figures are

liver in this way must leave it in the bile, and that probably a fairly

bile.

D. A. Long: Guinea pigs with extensive cirrhosis of the liver secondary to protein deficiency produced antitoxin more rapidly and in larger amounts than controls.

test and PBI determination on patients with liver cirrhosis. The results of these experiments in ten cases of liver cirrhosis show that iodine fixation in the thyroid and the conversion rate are practically normal. The PBI is a little increased, but not statistically significant. We also found a clear difference between the results obtained in infectious hepatitis and those in liver cirrhosis.

We think that the difference in the iodine metabolism between infectious hepatitis and liver cirrhosis is dependent on the fact that, in infectious hepatitis, the liver damage is acute and diffuse on all the liver parenchyma. In the liver cirrhosis, there are still some liver cells which have a practically normal function and which could partially compensate for the normal function of the liver cell in thyroxine metabolism.

D. A. Long The livers we used were sectioned and there was extensive

liver slices (R_F 0·7–0 9)

This spot contains lipids. Extraction with lipid solvent and elective coloration with Sudan show the spot to contain ten times more lipid than the thyroxine spot. It also contains inorganic iodine.

The butanol extracts of the PBI contain a certain quantity of this iodine.

We suspect that we are not dealing with a special iodine compound but rather an iodine-lipid association or with an artifact.

Wilkinson: In making butanol extracts of solutions of either triiodothyronine and thyroxine, we have on many occasions found a fast-running spot, and as that had a considerable bearing on the work which I summarized yesterday, we worked very hard on the problem to find out the cause. One of my colleagues, Mr. Bowden, subsequently found that this fast-moving spot was due to the butyl ester of thyroxine or triiodothyronine. This was confirmed by treatment of the solution with alkali and the fast-moving spot disappeared and the thyroxine or [illegible] Subsequently, he treated thyroxine [illegible] . cid butanol and obtained sufficient [illegible] Moreover, it did not seem to matter how much water was present in the acid butanol, esterification still occurred.

I wonder whether, in your experiment, the plasma extract from the patient who had a high dose of radioactivity was perhaps treated a little more delicately and was left in contact with acid butanol for a longer period than usual.

ing [illegible]
of [illegible]

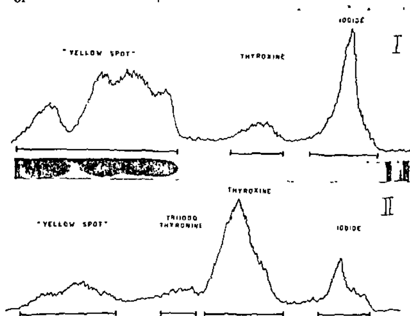


FIG. 1 (Vannotti) Chromatograms of butanol extracts of patients who have received 14 mc ^{131}I.

I. 3 hours later; elective coloration of "yellow spot" with Sudan.

II. 9 hours later; increase of thyroxine, small spot of triiodothyronine, and decrease of "yellow spot" and iodides.

cholesterol, in the transport and the fixation of iodine and iodine compounds in the body.

P.-Rivers: I must say I think Dr. Wilkinson's explanation of this is much the most likely. I cannot think why we did not think of this before. Butyl esters are formed with extraordinary ease even in the presence of large amounts of water.

Taurog: What about the pH effect? There is more in acid than in alkali.

P.-Rivers: Yes. I think if you made your extract alkaline before concentrating it esterification would not take place.

Maclagan: As regards this yellow spot, perhaps I ought to make the point that the butyl ester is, of course, not a yellow spot.

Taurog. There is the possibility of adding a reducing agent, like thiouracil, before you do your chromatography. If it is due to the

in most solvent systems, and the amount of that material which *crops* up appears to be related to the amount of haemolysis.

P.-Rivers We have had that too—a rather pinkish hue.

Wilkinson: Yes.

Vannotti: In our experiments we could exclude the possibility of an influence of haemolysis on the production of the iodine spot.

THYROID HORMONES AT THE PERIPHERAL TISSUE LEVEL: METABOLISM AND MODE OF ACTION

ODETTE THIBAULT

Laboratoire de Physiologie Générale, Université de Paris

SINCE the isolation of the active principle of the thyroid by Kendall, extensive experimental work has been carried out on its formation in the gland, its liberation into the blood stream and the regulation of its secretion through the pituitary gland, but very little is known concerning the mode of its peripheral action.

The object of our study is to examine to what extent the *activity of the thyroid hormones may be determined or even* conditioned by their metabolism through the peripheral tissues themselves. It is not our intention to summarize all the known facts in a complete general review. All we want is to settle the problem by considering the most recent data on which is based the conception we are going to describe.

How does the problem of the active form of the hormone at the periphery present itself?

As has been known since the first experiments on the effect of thyroxine on the organism, no action becomes evident until after a delay of several hours. That fact is very striking when we compare it with the well-known rapid effects of adrenaline. That "latent period", as it was called, has been a handicap for physiologists wishing to study the enzymic mechanism of the catalytic action of thyroxine; it renders, indeed, quite impossible any experimentation *in vitro* on isolated tissues, as the hormone is inactive under those conditions and has to pass through the living organism before inducing its characteristic effects. So, the numerous attempts made in that way (*see* Barker, 1951) have always given inconclusive results.

What could be the significance of that latent period? It was first assumed to correspond to the formation of a complex molecule resulting from a combination of thyroxine with a protein, such as thyroglobulin; but in his general review Harington (1945) pointed out that such an idea would only constitute an unnecessary complication. It has nevertheless been maintained by some physiologists (for instance, Abelin and Huber, 1951).

Among other hypotheses, it could be thought that thyroxine might exert an indirect action through the intermediary of the pituitary, especially since Courrier and his co-workers (1951) have shown an accumulation of thyroxine in the posterior pituitary; but we have seen, on the other hand (Thibault, 1954, unpublished), that dibenamine which is capable of blocking the discharge from the anterior pituitary (Everett and Sawyer, 1949) does not modify the activity of thyroxine. We also thought of the possibility that the injected hormone might be stored in the liver in some "hidden form" and be liberated progressively into the circulation; but partial or incomplete hepatectomy does not modify (Thibault, 1954, unpublished) or slightly enhances (Grad and Leblond, 1950) the increase of respiratory exchanges occurring after administration of normal doses of thyroxine: and in no cases does it modify the latent period (Thibault, 1954, unpublished). That possibility has thus been eliminated. We will see later on how the regulating rôle of the liver may be interpreted in the light of recent developments.

As no physiological interpretation seems to be satisfactory, we tried to find out whether the latent period might be due to a *chemical* transformation of thyroxine from an inactive into an active compound, the nature of which will have to be determined.

The discovery of triiodothyronine, the most important one in the field of thyroid physiology during the last few years, brought the hope of finding the solution to the problem. After its characterization in plasma and the study of its physiological properties by Gross and Pitt-Rivers (1952) we

thought, together with these authors and others, that triiodothyronine might be the active form for which we were looking. But as we shall see, such is not the case.

During the last few years, the study of triiodothyronine has been carried out on a large scale; the contributions are so numerous that we shall not review them here (*see* Barker's review, 1955*a*). It may suffice to say that, by the same standards as thyroxine, triiodothyronine can be called a "hormone". Both are formed in the gland and both pass into the circulation; both exert the same activities in all tests but in different degrees, the tri-substituted derivative showing most often the highest activity. But it has not been demonstrated yet that triiodothyronine is secreted after a given stimulus, moreover we do not know if it is discharged preferentially to thyroxine in some special cases where it depends on a specific thyroid function, as seems to be true for adrenaline and noradrenaline. It has only been shown that, in the gland, under certain conditions (iodine deficiency) triiodothyronine is preferentially synthesized (Leloup and Lachiver, 1955). Data allowing triiodothyronine to be placed exactly in thyroid physiology are, therefore, still lacking. The question is further complicated by the introduction of a third derivative—diiodothyronine (Roche, Michel, Nunez and Wolf, 1955) to which there is no reason to deny the name of "hormone" either.

Whatever it may be, the fact which interests us here is that the use of triiodothyronine instead of thyroxine does not in any way suppress the latent period, the duration of which is always approximately the same (Gross, Pitt-Rivers and Thibault, 1953). Furthermore, triiodothyronine does not exert more than thyroxine any immediate action on the respiration of tissue slices *in vitro* (Thibault, 1955*a*). Therefore triiodothyronine is not the active form at the peripheral tissue level, and the latent period does not correspond, as had been supposed, to the deiodination of thyroxine in the tissues. One can find another proof of this in Barker's latest data (1955*b*); indeed, if thyroxine were acting only after deiodina-

would be due to their inability to realize such a chemical change and, consequently, triiodothyrononine should act directly on them; but this is not the case, the same tissues are insensitive to both hormones.

The discovery of triiodothyronine did not enable us to resolve the question of the latent period, and three years of work with that compound can thoroughly be summarized by the following sentence of Klemperer (1955): "the latent period might, therefore, correspond to the formation of some active form of the hormone, different from both thyroxine and triiodothyronine".

What might that "active form" be?

The study of sensitization phenomena led us to consider the occurrence of a simple degradation in the side-chain of the molecule. This indirect effect of "sensitization" consists in an enhancement of the action of other hormones, especially adrenaline, or some pharmacological substances. With Lachaze (Thibault and Lachaze, 1951*a*, *b*; Lachaze and Thibault, 1951, 1952*a*, *b*) we were able to identify the compound acting in that phenomena: thyroxamine, resulting from the decarboxylation of the side-chain alanine in tissues during the latent period. The use of synthetic thyroxamine enabled us to reproduce all the sensitizing effects without the appearance of a latent period at concentrations as low as 10^{-14} (Thibault, 1951). We have also shown that triiodothyronine, as well as thyroxine, is able to produce those potentiating effects only after its transformation into triiodothyronamine (Harington and Thibault, 1953). On the contrary, the same "amine forms" were shown to be inactive on respiratory exchanges (Thibault and Lachaze, 1952; Thibault, 1952). We concluded that the amine function at the extremity of the side-chain is specifically responsible for this sensitization phenomena depending on thyroid hormones.

Another derivative might, therefore, be responsible for the direct catalysing action on cellular oxidations. After having shown that this effect develops in isolated tissues after

prolonged contact with the hormones at low temperature (Thibault, 1955*a*), we tried to determine what kind of transformation the hormones undergo during this lapse of time, and thought (by analogy with the decarboxylation which we spoke of above) of a simple deamination leading to the formation of the propionic acid derivative. However, this compound was found to be inactive on the respiration of tissue slices (Thibault, 1955*b*). At that time, our attention was drawn to the work of Pitt-Rivers who succeeded in synthesizing the acetic derivatives of the thyroid hormones (Harington and Pitt-Rivers, 1952; Pitt-Rivers, 1953); she showed that triiodothyroacetic acid given daily displays effects entirely analogous to those obtained with triiodothyronine. We then tested, in collaboration with Pitt-Rivers, the effects of the acetic derivatives on respiration of tissue slices and found that, in contrast to triiodothyronine and thyroxine, they produce an important and, what is more striking, an *immediate* effect (Thibault and Pitt-Rivers, 1955*a*, *b*). We consequently thought that thyroid hormones become calorigenically active after transformation into their acetic derivatives in tissues.

The study of these acetic derivatives has been extended to other tests. Let me cite: reversion of thiouracil-induced goitre in rats (Pitt-Rivers, 1953); restoration of the altered plumage of thyroidectomized birds to normal (Bruce, Pitt-Rivers and Sloviter, 1954); growth stimulation (Pitt-Rivers, 1955, unpublished); loss of weight and fall of blood cholesterol (Lerman and Pitt-Rivers, 1955); immediate stimulation of oxygen consumption and glycolysis in ascites tumour cells *in vitro* (Heimberg, Park, Isaacs and Pitt-Rivers, 1955); desensitizing action on tuberculin (Long and Pitt-Rivers, 1956).

From the point of view of basal metabolic rate (BMR) of the whole animal Pitt-Rivers has shown that daily administration of triiodothyroacetic acid at relatively high dosage enhances oxygen consumption in the male rat as does triiodothyronine. We have seen (Thibault and Pitt-Rivers, 1955*a*), in a first approximation, that a single injection has an

immediate effect. Lerman and Pitt-Rivers (1955) found no significant change in BMR or pulse rate in myxoedema. But Trotter (1955) found that if a single strong dose has no effect, the same given orally and fractionally in four or five injections raises the BMR in myxoedema.

Our own simultaneously conducted experiments on basal metabolism of thyroidectomized adult male rats confirm that result (Thibault, 1956*a*); we observed that too high doses are ineffective, or induce a contrary effect, as we had already seen *in vitro* (Thibault and Pitt-Rivers, 1955*b*), and that the effect reappears after fractionation. Now if these first results obtained by the introduction into the organism of a single dose of the activator proved that it was acting without any latent period, a fact of prime importance, they did not entirely satisfy us in that we could not obtain thereby more than a transient increase of 40 per cent. How would one explain, in such conditions, the slow development of the effect of an injection of thyroxine, which begins after 12 hours, reaches its maximal value after 48 hours, decreases slowly and returns to its initial level only after 5 or 6 days (Thibault, 1949)? In fact, under normal conditions we presume that the hormone discharges progressively at the effector level very small amounts of active derivative, the use of high single doses is, therefore, entirely artificial. In order to operate in physiological conditions, we elaborated a technique which permits a continuous injection of very small amounts of the acetic derivative into the animal, without taking it out of the metabolism box (Thibault, 1956*b*). It was thus possible to follow the effect as it gradually develops, the light anaesthesia needed in that kind of experiment does not cause any trouble (Thibault, 1956*b*).

We shall give a few examples of curves obtained by that procedure, which will facilitate the discussion of the mechanism of the observed phenomena (the whole experimental work will be published later in *Arch. Sci physiol.*).

The first, most striking fact which appears is the extreme sensitivity of the effector: 0·01 μg. injected every 1 or 2

minutes brings about an immediate increase of oxygen intake already starting during the first minutes of the injection (Fig. 1).

If the same frequency of injection is kept up during the whole experiment, the effect increases, then stabilizes and diminishes, which suggests that the rate of destruction of the activator is greater than that of its introduction. If the

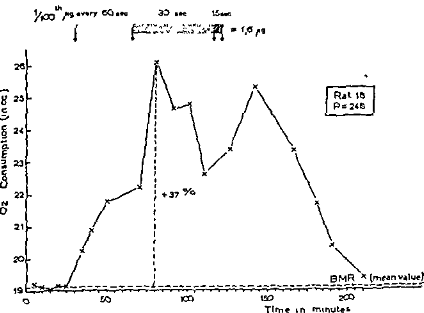


FIG. 1. *Immediate effect of very small quantities of TRIAC (here 0 01 μg), injected repeatedly and almost continuously into the caudal vein, on oxygen consumption of the male albino rat.*

frequency of injection is too great (every 30 seconds, for example) or the amount injected every minute too large, the effect starts and suddenly stops: there is a "blocking effect" (the same phenomena as already observed, Thibault and Pitt-Rivers, 1955*b*; Thibault, 1956*a*). In order to allow the increase to develop itself in a visible form in time, it is necessary to inject a small amount each time and to increase progressively and cautiously the frequency of injection. By taking a higher dose ($\frac{1}{16}$ μg.) and extending injections at a

progressively accelerated frequency, we tried to reach maximum response, but could never obtain more than 40 to 50 per cent. When this value is reached, even if the activator concentration increases, the curve flattens at a certain level, and we observed another small increase only after cessation of the injections, when part of the excess of activator has been destroyed (Fig. 2).

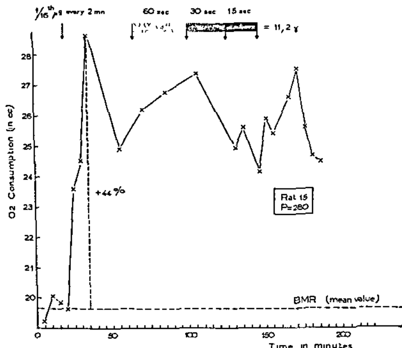


FIG. 2. Effect of TRIAC injected under the same conditions as Fig. 1., but at a higher dosage (1/16th μg)

It seems that above a certain "saturation state" the effector could not react any more.

On the other hand, it is possible to greatly diminish the effective dose injected: we observed a visible response with a dose as small as 0·001 μg. injected with progressively increasing frequency during 1 hour 45 minutes, the total amount

being 0·5 μg. One can consider that dose as the threshold dose: it is extremely small and demonstrates the great sensitiveness of the effector (Fig. 3).

We would point out that under the same conditions triiodothyronine remains entirely inactive.

We should keep in mind that it is not only impossible to obtain an increase higher than 40 to 50 per cent, but that this effect is not very lasting and disappears progressively and rather rapidly after the cessation of injections; this is in contradiction to the fact that daily injections of the hormone

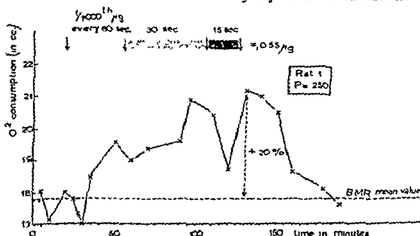


FIG 3 Effect of TRIAC at the threshold dose (0 001 μg.).

(as well as of its acetic derivative) may bring about increases of metabolism easily reaching 100 per cent after 8 days.

In order to explain that phenomenon we many times tried to repeat the same experiment on the same animal. As you can see (Fig. 4), if during the first day the effect stops at a value about 40 per cent and does not persist, it can on the second day increase further and last longer, in such a way that on the third day the metabolism has not returned to its initial value; further injections of the active derivative, adding their effects to the residual one render it possible to attain a 60 per cent increase and more in the basal metabolism value (Fig. 5).

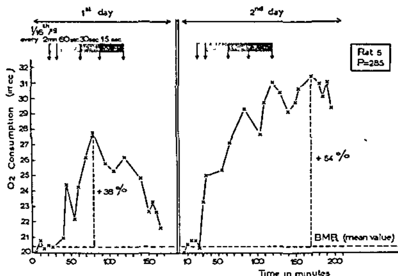


FIG 4. Effect of TRIAC in the same experiment repeated on two consecutive days on the same animal

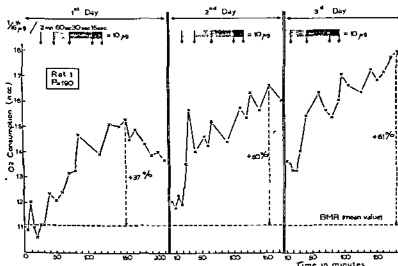


FIG. 5. Increase of effect of TRIAC day by day (same experiment on the same animal on 3 consecutive days)

Even medium-high doses which produce a rather small initial increase (as it is the case in Fig. 5) exert a long-lasting action on the second day and enable, by summation of small effects, a global important action to be obtained on the third day. It is impossible to continue after 8 days, because one cannot indefinitely repeat an experiment which necessitates anaesthesia on the same animal, but it is easily conceivable from these results that daily injections of hormone may, after a certain number of days, bring about a two-fold increase of the value of basal metabolism.

How can these results be interpreted? The absence of any effect, or even the slightly negative one of too high doses, is probably due to a toxicity phenomenon. In order to explain the daily increase of the response it is necessary to imagine a progressive adaptation of the reactive systems and, gradually with the discharge of the activator at cellular level, either a supply or a synthesis of enzymes or any element whose presence is necessary for the development of the response. In that connection it may be interesting to consider the data of Smith and Williams-Ashmann (1951): these authors noticed an increase in the concentration of certain enzymes during prolonged administration of thyroxine. It is far harder to suppose that the effect of the activator is getting more and more lasting. Can we suppose that its speed of destruction diminishes progressively as it is included in the chain of enzymic reactions leading to the increase of oxidation? All this belongs to the realm of hypothesis. All we can say, starting from what we know, is that the use of acetic derivatives of the hormones gives an exact account of the slow and progressively increasing evolution of metabolism under their influence. Such a mode of action finds its explanation in a slow and continuous discharge of small amounts of the active derivative (as we tried to represent above) at the effector level which, by means of an adaptation, attains a more and more reactive state.

Another phenomenon which becomes more and more certain is the presence of a regulator mechanism by which the

hormone, when introduced in excess, is eliminated. We can see proof of this in the fact that if the injection of 1 mg. thyroxine/kg. daily produces 100 per cent increase after 8 days, the injection of a daily dose 10 times larger does not exert a stronger effect. This regulatory system could depend on the liver; storing and elimination of the surplus in the bile in the form of glycuro-conjugates, returned by the entero-hepatic circulation. This idea, advanced by several authors (Grad and Leblond, 1950; Klitgaard, Lipner, Barker and Winnick, 1953; Taurog, Briggs and Chaikoff, 1952) seems to be definitely proved correct by Roche and co-workers (1954).

In conclusion, it appears from our study on the metabolism *in vivo* that acetic derivatives of thyroid hormones immediately increase oxygen consumption of the whole animal. Those are the only derivatives having such a property. Moreover, their use under conditions closest to physiological ones makes it possible exactly to reproduce the effects of thyroid hormones but without any latent period These data, together with those obtained *in vitro* (Thibault and Pitt-Rivers, 1955*a*, *b*) point to the conclusion that the hormones from the thyroid gland exert their calorigenic action in the form of the "acetic acid". No other explanation of this immediate effect of these substances appears as valid to us. The objection that the faster action of acetic derivatives may be due to the fact that they increase membrane permeability and, consequently, enter faster into the cells (Hoch and Lipmann, 1954) could be accepted for experiments carried out *in vitro* on tissue slices, but such an explanation cannot be put forward to explain a difference in speed of action of 12 hours *in vivo*. On the other hand, it has been demonstrated even *in vitro* that the delayed effect of hormones is really due to the formation of an active derivative during an incubation period (Thibault, 1955*a*), and not to difficulties of penetration.

In order to be quite sure that the thyroid hormones act at the peripheral tissue level as the acetic derivatives, it remained to demonstrate the occurrence of such compounds in tissues after the introduction of hormones. Such an occurrence has

been recently found by Roche and co-workers in the kidney (Roche, Michel, Jouan and Wolf, 1955) and in muscle (Roche, Michel and Jouan, 1956).

If we really know the substance directly responsible for the catalysis of oxidation, it is now possible to study the enzymic mechanism of that phenomenon. The experiments of Le Breton and Le Van Hung (1956) may open a way by showing the rôle of coenzyme A in the action of triiodothyroacetic acid.

Discussion and Conclusions

It appears, therefore, that the latent period can be explained by the necessity for thyroid hormones to undergo chemical transformations which render them capable of exerting their peripheral action. It is remarkable how simple they are; they require enzymes normally present in almost all tissues. It has been known for a long time that through the interplay of amino acid oxidase all aromatic amino acids can be transformed into the corresponding acetic acids. Decarboxylation processes by which an active derivative is formed can take place in all tissues (Lachaze and Thibault, 1952*a*). Some of them, on the contrary (spleen, uterus) which are rich in decarboxylase (Lachaze and Thibault, 1952*a*) are probably poor in enzymes endowed with the power of transforming the side-chain *alanine into acetic acid, as these tissues are not sensitive* to hormones injected *in vivo* (Barker, 1955*b*)

Thus it appears that, by the normal action of tissue enzymes, an inactive hormone becomes active by simple modification of the side-chain of its molecule. These changes are probably not the only possible ones in peripheral tissues; modification of the nucleus can also occur: the possibility of a deiodination of thyroxine is still under discussion at present (Gross and Pitt-Rivers, 1953; Roche, Lissitzky and Michel, 1953; Wilkinson and Maclagan, 1954; Albright, Larson and Tust, 1954; Flock and Bollmann, 1954; Kalant, Sellers and Lee, 1954; Hogness, van Arsdel and Williams, 1954; Sprott and

Maclagan, 1955) but it is not impossible that, since deiodinases exist in tissues, the change from thyroxine to triodothyronine takes place at the peripheral level. It is also possible that both kinds of modification (degradation of the side-chain and substitution on the nucleus) are linked together: in fact, Hartmann (1950) has pointed out that 3 : 5-diiodo-4-hydroxyphenylpyruvic acid loses its iodine spontaneously in aqueous solution. Roche and Michel (1955) have examined the possibility that "deiodination takes place only after degradation of the alanine residue, giving rise to substances in which the halogen bond has become very labile". Whatever the explanation may be, the number of halogen bonds in the nucleus do not in any way influence the latent period and it is the modification of the side-chain which determines and conditions the sense of activity (sensitization or oxidation catalysis). Reactions taking place at the peripheral level are therefore extremely important

This leads us to revise our conception on the effector, which is not only an organ reacting passively under the influence of the hormone, but also the seat of an enzymic activity capable of realizing chemical changes and conferring their activity upon the hormones.

We have just shown from experimental facts to what extent hormonal metabolism at the peripheral level determines and even conditions hormonal activity. We are thus obliged to modify our first idea on thyroid hormones: first of all, the very term "hormones" should be restricted to the circulating forms (which are two at least) They are themselves transformed, in tissues, not only into one but into several active derivatives. To that notion of multiplicity of active forms is necessarily linked the specificity concept. We have seen that if thyroxamine exerts a highly efficient sensitizing activity, it does not act on respiratory exchanges, the contrary is true for acetic derivatives. The amine function is, consequently, specific of sensitization, the acetic acid function of oxidation catalysis. Is it possible further to develop that idea and to suppose the propionic derivative to be specifically responsible

for metamorphosis phenomena? In fact, it appears extremely active in that mechanism (100 to 1000 times greater than thyroxine according to the following authors: Bruice, Winzler and Kharasch, 1954; Roth, 1955; Roche, Michel, Truchot and Wolf, 1955) though it is absolutely inactive on respiration *in vitro* (Thibault, 1955*b*) and *in vivo* (Thibault, 1956, unpublished).

In conclusion, thyroid hormones carried by the blood into tissues could be transformed therein into a certain number of active derivatives each of them being specific of a particular function.

Acknowledgements

We wish to express our best thanks to Odette Hesse for her technical assistance, to Mr. Goldstein for having kindly undertaken the translation of this communication, and to Dr. R. Pitt-Rivers for the sample of TRIAC she synthesized and sent us, and which enabled us to carry out these experiments.

REFERENCES

ABELIN, J., and HUBER, P. (1951). *Acta endocr.*, 6, 1.

ALBRIGHT, E. C., LARSON, F. C., and TUST, R. H. (1954). *Proc. Soc. exp. Biol., N.Y.*, 86, 137.

BARKER, S. B. (1951). *Physiol. Rev.*, 31, 205.

BARKER, S. B. (1955*a*). *Ann. Rev. Physiol.*, 17, 417.

[illegible]

[illegible] 954). *J.*

[illegible]

[illegible] *l. Chem.*, 210, 1.

COURRIER, R., HOREAU, A., MAROIS, M., and MOREL, F. (1951). *C. R. Acad. Sci., Paris.*, 232, 776.

[illegible] *Endocrinology*, 44, 234.

[illegible] 13, 209.

[illegible] *ol*, 162, 17.

[illegible] 9, 593, 1044.

[illegible] 3, 645, 652.

[illegible] *C. R. Soc. Biol.*,

[illegible]

HARINGTON, C. R. [illegible]

HARINGTON, C. R., and PITT-RIVERS, R. (1952). *Biochem. J.*, 50, 438.

HARINGTON, C. R., and THIBAULT, O. (1953) *C. R. Soc. Biol., Paris*, 147, 78.

HARTMANN, N. (1950). *J. physiol. Chem.*, 285, 105.
HEIMBERG, M , PARK, J. H , ISAACS, A., and PITT-RIVERS, R. (1955). *Endocrinology*, 57, 756.
HOCH, F. L., and LIPMANN, F. (1954). *Proc. nat. Acad. Sci., Wash.*, 40, 909.
HOGNESS, J. R., VAN ARSDEL, P., and WILLIAMS, R. H. (1954). *J. clin. Endocrin. Metab.*, 14, 772.
KALANT, E. H , SELLERS, A., and LEE, R. B. (1954) *Fed. Proc.*, 13, 76.
KLEMPERER, H. G. (1955). *Biochem J.*, 60, 122, 128.
KLITGAARD, H. M , LIPNER, H. J., BARKER, S. B , and WINNICK, T. (1953). *Endocrinology*, 52, 74
LACHAZE, A., and THIBAULT, O. (1951) *Bull. Soc. chim. Biol.*, 33, 1456, 1458.
LACHAZE, A., and THIBAULT, O. (1952*a*). *C. R. Soc. Biol , Paris*, 146, 50
LACHAZE, A , and THIBAULT, O (1952*b*). *C. R. Soc. Biol., Paris*, 146, 393.
LE BRETON, E., and LE VAN HUNG. (1956) *C. R Acad. Sci , Paris*, 242, 1357.
LELOUP, J., and LACHIVER, F. (1955) *C R Acad Sci., Paris*, 241, 509
LERMAN, J , and PITT-RIVERS, R. (1955) *J. clin. Endocrin Metab* , 15, 653
LONG, D. A., and PITT-RIVERS, R (1956). Unpublished, *see* Pitt-Rivers, R. (1956) IIIrd Congr Biochem , Brussels
PITT-RIVERS, R. (1953). *Lancet*, 1, 234
ROCHE, J , LISSITZKY, S , and MICHEL, R. (1953). *Biochim. biophys. Acta*, 11, 220.
ROCHE, J , MICHEL, O , MICHEL, R., and TATA, J. (1954). *Biochim. biophys. Acta*, 13, 471.
ROCHE, J., and MICHEL, R. (1955). *Physiol Rev* , 35, 583.
ROCHE, J., MICHEL, R , and JOUAN, P (1956). *C R. Soc. Biol , Paris*, 150, 629.
R[illegible] and WOLF, W. (1955). *C. R. Acad.* [illegible]
I[illegible] WOLF, W. (1955) *C. R. Soc.* [illegible]
ROCHE, J , MICHEL, R , TRUCHOT, R , and WOLF, W. (1955). *C. R. Soc. Biol., Paris*, 149, 1219.
ROTH, P. (1953) *C. R. Soc. Biol., Paris*, 149, 1180.
SMITH, R. H., and WILLIAMS-ASHMANN, H. G. (1951). *Biochim. biophys. Acta*, 7, 295.
SPROTT, W E , and MACLAGAN, N. F (1955) *Biochem. J.*, 59, 288.
TAUROG, H., BRIGGS, F N., and CHAIKOFF, I. L (1952). *J. biol. Chem.*, 194, 655.
THIBAULT, O. (1949). *Rev. canad. Biol.*, 8, 3
THIBAULT, O. (1951). *Ann Endocr., Paris*, 12, 674.
T[illegible]
T[illegible]
T[illegible]
T[illegible]
T[illegible]

THIBAULT, O., and LACHAZE, A. (1951a). *C. R. Acad. Sci., Paris*, 232, 1318.
T ... R. *Soc. Biol., Paris*, 145, 707.
... *R. Soc. Biol. Paris*, 146, 393.
... *Lancet*, 1, 285.
... *C. R. Soc. Biol., Paris*, 149,
TROTTER, W. R. (1955). *Lancet*, 2, 374.
WILKINSON, J. H., and MACLAGAN, N. F. (1954). *Biochem. J.*, 56, 7.

DISCUSSION

Barker: Although I should like to agree with the very beautiful con-

response of the acetic acid derivatives. I have already mentioned our study of the metabolic action of several fatty acid side-chain analogues of thyroxine (*see* p. 166). I quite agree that some of these are turning out to have some very peculiarly specific effects, as on metamorphosis

Our experience with the sensitization of uterine and intestinal smooth muscle to epinephrine inhibition or stimulation in different species has been that this is a phenomenon which one can obtain under certain circumstances with triiodothyronine, with thyroxine, or with some of their analogues. We have been quite unable to check the thyroxamine effect on smooth muscle sensitization because we kill our tissue with either the glacial acetic acid or the propylene glycol required to dissolve the insoluble amine. Adding a fine suspension of the amine to the tissue is without effect.

I should like to propose a counter-theory, that instead of having a single, instantly active form of the hormone, we actually have enzymic changes produced in the tissues by thyroactive substances accounting for the latent period. So far we have not found any of the thyroxine analogues active without a latent period. I should add that in the thyroidectomized animal we find that heart, which is an extremely sensitive tissue, responds about 6–12 hours earlier than the other tissues to triiodothyronine, TRIAC and TETRAC, but not thyroxine. But this again does not, in my opinion, show a specific action for the acetic acid derivatives.

Thibault. It may be a variation of the sensitivity among the tissues.

removed the tissues for study.

Thibault:

Barker: D

whole anima

similar inabi

Thibault: At first, we were unable to get satisfactory results by injecting the acetic acid derivative *in vivo*. But here we have a good dose and mode of administration.

Lardy: I think Dr. Asper's Endocrine Society Abstract dealt with patients rather than with animals

can easily miss it.

Barker: We ran Warburg experiments with rates of shaking down as low as 60 per minute where the rate of shaking with kidney is critical and up to 120, without effect.

Thibault: It may be due to that phenomenon of adaptation in time which can occur in whole animals and not in tissue slices

Lardy: If you give a single injection of the acetic acid analogue on each of three successive days, do you observe the adaptive response in basal metabolic rate?

Thibault: I have not tried that.

Lardy: If you do it with a propionic acid derivative you get an increase in basal metabolic rate? I think that you must not discount the propionic analogue for basal metabolism because it is really a rather effective hormone in the rat If it is injected once a day for a period of a week, you get a very good response, about half as great as with an

experimental conditions, where

ive, i e *immediately*. It is not impossible that it became active by repeated daily injection and not quite impossible to think that we can pass from one to another derivative in the organism

The object of my search is the latent period. There are plenty of derivatives that are active by repeated daily injection, but the only point which interests us is the disappearance of the latent period, and I think it is the only case (acetic derivatives) in which the latent period disappears in basal metabolism.

THIBAULT, O., and LACHAZE, A. (1951*a*). *C. R. Acad. Sci., Paris*, 232, 1318.
… *R. Soc. Biol., Paris*, 145, 797.
… *R. Soc. Biol. Paris*, 146, 393.
… *Lancet*, 1, 285.
… *C. R. Soc. Biol., Paris*, 149, 880.
TROTTER, W. R. (1955). *Lancet*, 2, 374.
WILKINSON, J. H., and MACLAGAN, N. F. (1954). *Biochem. J.*, 56, 7.

DISCUSSION

Barker: Although I should like to agree with the very beautiful concept that Mme Thibault has presented, as some of you know we have been quite unable to confirm many of the qualitative findings. Much search has failed to reveal any evidence for an immediate metabolic response of the acetic acid derivatives. I have already mentioned our study of the metabolic action of several fatty acid side-chain analogues of thyroxine (*see* p. 166) I quite agree that some of these are turning out to have some very peculiarly specific effects, as on metamorphosis.

Our experience with the sensitization of uterine and intestinal smooth muscle to epinephrine inhibition or stimulation in different species has been that this is a phenomenon which one can obtain under certain circumstances with triiodothyronine, with thyroxine, or with some of their analogues. We have been quite unable to check the thyroxamine effect on smooth muscle sensitization because we kill our tissue with either the glacial acetic acid or the propylene glycol required to dissolve the insoluble amine. Adding a fine suspension of the amine to the tissue is without effect.

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to make serial BMR readings over the period 4–16 hours without

of only 4 mg. given orally.

Thibault: With big doses, I obtained nothing on the rat; there is a blocking effect.

Fraser: Figure 3 shows a contrast which should be borne in mind. This concerns a patient who was completely myxoedematous at the time of the test and was given an oral dose of TRIAC of the same order as the

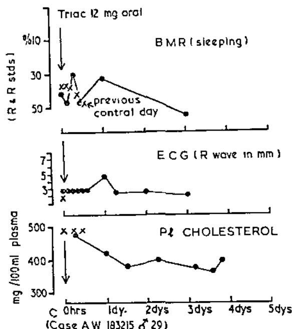


FIG. 3 (Fraser). Acute effects of TRIAC (untreated complete myxoedema).

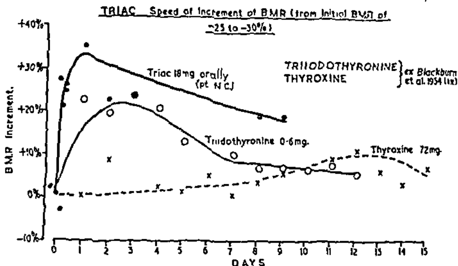


Fig. 1 (Fraser). TRIAC: Speed of increment of BMR (from initial BMR of −25 to −30%)

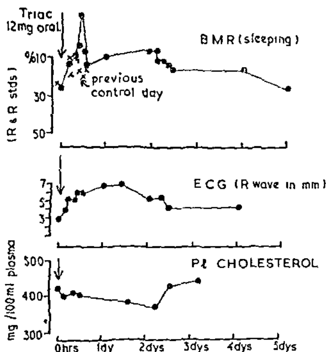


Fig. 2 (Fraser). Acute effects of TRIAC (Complete myxoedema + hypopituitarism; 27 days after thyroxine)

enzymes in the tissues Smith and Williams-Ashman have noticed an increase in the concentration of certain enzymes during prolonged

it concerns quite a different enzymic mechanism.

Fraser: You used thyroidectomized animals for these experiments?

Thibault: Yes.

Fraser: And they had no thyroid administration preceding your experiments at all?

Thibault: No.

Fraser: We wondered, I might say, whether the other patient that I showed you failed to have an acute BMR effect because he had no thy-

pletely thyroid-less animal needs to be primed up with thyroid hormone before you can get an acute BMR effect from TRIAC, because the penetration into the cell is so much slower in a completely thyroid-deficient animal

B.-Grant: To return to Dr. Gross's point, as I understood him yester-

the speed of triiodothyronine.

B.-Grant: What about the accumulation in muscle, then?

Michel It is also the same.

injected daily for 8 days is 1 mg /kg , or 10 mg /kg , it is exactly the same in the case of TRIAC as in the case of triiodothyronine. So I

previous patient. Here there are scarcely any BMR or ECG effects, but I think there is not much doubt—perhaps Dr. Barker would agree—about the cholesterol ... ted at first only with plasma choleste ... atous, while the other one, ... weeks

... ether—the three compartments ... y conclusions about the relative latent period with such tremendous variations in doses. You show here ... maximal BMR for many, many days. It ...

roxine. So I think conclusions could ... equivalent doses of triiodothyronine or thyroxine in comparison with TRIAC.

Fraser: I agree it may be a question of dosage. But it certainly is an ... whether it is due to dosage or not.

... propionic acid?

ronine in the same ... y from the ... l expect?

Michel: ... tion of the exc ... er ho ... ne is ... ve ... al in

I ... It is the same as triiodo ... that ... op- ... of

CELLULAR ACTIONS OF THYROXINE AND SIMILAR COMPOUNDS*

S. B. Barker

Department of Pharmacology, University of Alabama Medical Center, Birmingham, Alabama

In trying to find an answer to the riddle of the mechanism of action of the thyroid hormone, workers in the field have clutched at whatever straw seemed to offer some support. Like the classical blind men inspecting the mysteries of an elephant, we have each felt for some particular response and considered it to be the one most crucial to hormonal action. The purpose of this paper is not to decide what is the most likely site of action of the hormone, but simply to present a progress report on some of our most recent endeavours.

A primary problem is still what is to be considered as *the* thyroid hormone. Here, may I touch again on a point which has been made before in these discussions: I feel that there may well be a series of physiological thyroactive compounds, depending upon circumstances which are at present poorly evaluated. Triiodothyronine (TRIT or T_3) is without question, at least in mammals, the most active one of a long series, but we still lack much basis for labelling TRIT as the only active form of the thyroid hormone. A real challenge for future investigation lies in the observations that the propionic acid analogue of thyroxine has such a tremendously greater effect on amphibial metamorphosis than even TRIT (Bruice, Winzler and Kharasch, 1954).

A wide variety of test objects has been used in evaluating thyroxine action, and I think it only fair to point out that just because something less than a whole animal has been employed

* This work was made possible by grants-in-aid from the Smith, Kline and French Foundation and the American Cancer Society.

think it would perhaps be an explanation, but it hardly explains the difference of 12 hours *in vivo*; and, moreover, there are our experiments, Dr. Barker's and mine, with incubation of tissue slices at low temperature. Dr. Barker, do you agree with my idea that during this incubation

port this rough extract onto a new and fresh tissue slice, I see an immediate effect—not considerable but immediate.

Thibault: That I have not tried.

Barker: We have; we have tried the tissue and the medium. We have extracted with saline and methanol and butanol, but we could find no evidence for anything giving an immediate effect.

several years, I have collected various bits of data indicating thyroxine effects on incubated mammalian tissues. Most of these were sufficiently vague as to be non-interpretable, because of the rapidly failing oxygen consumption of the control tissue. For instance, kidney slices held at 37° in serum for 12–18 hours consumed oxygen at least 50 per cent more rapidly in the presence of thyroxine than in its absence. Considering that the control tissue, without added thyroxine, had fallen to about 50 per cent of its starting rate, this "stimulation" could hardly be impressive.

A clue to a possible improvement in incubation technique was furnished by Thibault (1955), who discussed incubation of kidneys slices at 5° C between oxygen consumption determinations. Using this general procedure, we have obtained striking and consistent effects of thyroxine on oxygen consumption of isolated tissue. This approach may lead to a better understanding of thyroxine action.

The experiments to be discussed here are based on the conventional determination by the Barcroft-Warburg technique of oxygen consumption of thyroidectomized rat kidney cortex slices. An initial run was made at 37° over about one hour. The vessels were then capped with Parafilm and stored in a cold room at 5° C without shaking. At one day intervals, the vessels were opened, flushed with oxygen and the oxygen consumption redetermined at 37° after a 15-minute warming and equilibration period.

Thyroxine, or one of its analogues, was dissolved in a small amount of alkali, and diluted to a final concentration (in the flasks) of 0·0005 N-NaOH, with the addition of sufficient 10 per cent NaCl to make the solution isotonic with 0·9 per cent NaCl. Control flasks (referred to as "blanks") received an equivalent volume of identical solutions without the thyroxine. All solutions were freshly prepared at the time of setting up an experiment. All glassware and equipment, including that used in the preparation, weighing and delivery of tissue, had been scrupulously cleaned. However, no extraordinary aseptic precautions were taken, except that the vessels were

this does not necessarily imply that one has come closer to how *thyroxine acts. In this laboratory, we have had specific* experience (Barker and Lewis, 1956*a*) with the action of thyroxine and many of its analogues on the rapidly failing succinoxidase system devised by Gemmill (1952) and further studied by Asper *et al.* (Wiswell, Zierler, Fasano and Asper, 1954). There is no doubt about the tremendous enhancement of activity shown by thyroxine, as well as by many of its analogues, without essential regard to their metabolic effects in animals. Furthermore, several compounds which are not thyroactive in the thyroidectomized mammal, a truly sensitive test object, are just as active against the broken cell "succinoxidase" system. The pertinent suggestion of Clarke and Ball (1955) that the succinoxidase enhancement is operating through inhibition of malic dehydrogenase, thus decreasing production of oxaloacetate, a potent inhibitor of succinic dehydrogenase, has been confirmed. This, however, is also subject to the same criticism of non-specificity, structurally speaking.

In contrast to these *in vitro* functions, which are confirmable but which *seem to lack specificity, are other claims* which are equally dramatic but not duplicatable. Our own experience deals especially with the recent excitement over the acetic acid analogues of thyroxine and TRIT, namely TETRAC and TRIAC. *In contrast to claims of special activities of these* two analogues (Thibault and Pitt-Rivers, 1955), *they seem to* fit in well with the entire series of fatty acid analogues as thyroactive materials without notable qualitative differences (Barker and Lewis, 1956*b*). Actually, *I feel that the* mere fact of 75 per cent of thyroxine activity *being shown* by fatty acid rather than amino acid derivatives has considerable significance in the evaluation of *mechanisms of* action.

As emphasized before, a major obstacle in obtaining consistent responses to thyroxine in isolated systems is the rapidity with which most such biological preparations deteriorate. In collaboration with several *others in our laboratories over*

several years, I have collected various bits of data indicating thyroxine effects on incubated mammalian tissues. Most of these were sufficiently vague as to be non-interpretable, because of the rapidly failing oxygen consumption of the control tissue. For instance, kidney slices held at 37° in serum for 12–18 hours consumed oxygen at least 50 per cent more rapidly in the presence of thyroxine than in its absence. Considering that the control tissue, without added thyroxine, had fallen to about 50 per cent of its starting rate, this "stimulation" could hardly be impressive.

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this does not necessarily imply that one has come closer to how thyroxine acts. In this laboratory, we have had specific experience (Barker and Lewis, 1956*a*) with the action of thyroxine and many of its analogues on the rapidly failing succinoxidase system devised by Gemmill (1952) and further studied by Asper *et al.* (Wiswell, Zierler, Fasano and Asper, 1954). There is no doubt about the tremendous enhancement of activity shown by thyroxine, as well as by many of its analogues, without essential regard to their metabolic effects in animals. Furthermore, several compounds which are not thyroactive in the thyroidectomized mammal, a truly sensitive test object, are just as active against the broken cell "succinoxidase" system. The pertinent suggestion of Clarke and Ball (1955) that the succinoxidase enhancement is operating through inhibition of malic dehydrogenase, thus decreasing production of oxaloacetate, a potent inhibitor of succinic dehydrogenase, has been confirmed. This, however, is also subject to the same criticism of non-specificity, structurally speaking.

In contrast to these *in vitro* functions, which are confirmable but which seem to lack specificity, are other claims which are equally dramatic but not duplicatable. Our own experience deals especially with the recent excitement over the acetic acid analogues of thyroxine and TRIT, namely TETRAC and TRIAC. In contrast to claims of special activities of these two analogues (Thibault and Pitt-Rivers, 1955), they seem to fit in well with the entire series of fatty acid analogues as thyroactive materials without notable qualitative differences (Barker and Lewis, 1956*b*). Actually, I feel that the mere fact of 75 per cent of thyroxine activity being shown by fatty acid rather than amino acid derivatives has considerable significance in the evaluation of mechanisms of action.

As emphasized before, a major obstacle in obtaining consistent responses to thyroxine in isolated systems is the rapidity with which most such biological preparations deteriorate. In collaboration with several others in our laboratories over

several years, I have collected various bits of data indicating thyroxine effects on incubated mammalian tissues. Most of these were sufficiently vague as to be non-interpretable, because of the rapidly failing oxygen consumption of the control tissue. For instance, kidney slices held at 37° in serum for 12–18 hours consumed oxygen at least 50 per cent more rapidly in the presence of thyroxine than in its absence. Considering that the control tissue, without added thyroxine, had fallen to about 50 per cent of its starting rate, this "stimulation" could hardly be impressive.

A clue to a possible improvement in incubation technique was furnished by Thibault (1955), who discussed incubation of kidneys slices at 5° C between oxygen consumption determinations. Using this general procedure, we have obtained striking and consistent effects of thyroxine on oxygen consumption of isolated tissue. This approach may lead to a better understanding of thyroxine action.

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a 4 per cent solution. This actually caused a more rapid decrease in oxygen consumption, as did also neutralized rat serum and neutralized rat serum added to phosphate glucose. Several different concentration ranges were tried, although not shown in the figure, without success.

The next variation tried was the buffer, as shown in Figure 2. Although a definite effect of thyroxine was still present in tris(hydroxymethyl)methylamine-hydrochloric acid buffer, actual maintenance was much poorer than with phosphate.

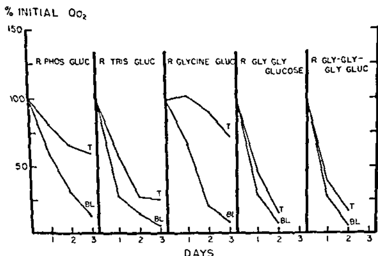


FIG. 2. Oxygen consumption of thyroidectomized rat kidney slices incubated in the various solutions shown. Metabolism determinations done at 37° C, incubation at 5° C without shaking.

When glycine-NaOH was used, control oxygen consumption was about the same as with phosphate, but with thyroxine it was as good as the starting level for at least 24 hours, and only fell off 9 per cent by 48 hours. This appeared to be less a function of any buffering ability of the glycine than some specific action of the amino acid, since glycylglycine and diglycylglycine, both more powerful buffers than glycine, gave poorer results.

heat-sterilized as part of the routine cleaning. Cultures of several flasks, made by Dr. C. H. Winkler of the Department of Microbiology, indicated less than 500 organisms per ml. even after 3 days of incubation.

Results and Discussion

As can be seen from Figure 1, when Krebs' Ringer phosphate glucose was used for incubation, the control tissue respiration

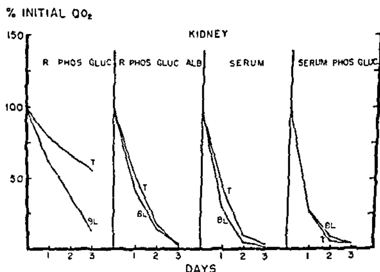


FIG. 1. Oxygen consumption of thyroidectomized rat kidney slices incubated in the various solutions shown Metabolism determinations done at 37° C, incubation at 5° C without shaking.

(BL) fell to 62, 38 and 13 per cent of the starting rates at 24, 48 and 72 hours, respectively. In the presence of 5 μg. L-thyroxine per ml. fluid, the corresponding values (T) were 79, 67 and 56 per cent. This considerable difference indicates some action of the thyroxine, but should not be termed a stimulation. Because of the possibility that colloid osmotic changes might account for the falling off, bovine serum albumin, electrophoretically free of globulins, was added to make

only slight liberation of the free alanine during the incubation with the acetylated form.

To find out more in detail about the changes taking place early in incubation, readings were taken as indicated in Figure 4. It should be realized that more frequent readings require more time at 37° in proportion to the total incubation at 5°, and may alter experimental conditions. With kidney in

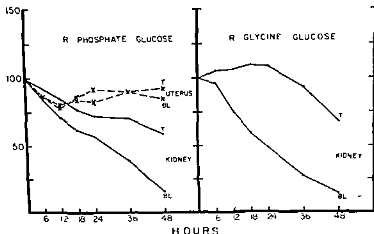


FIG. 4. Comparison of Ringer glycine glucose and Ringer phosphate glucose as incubation media for kidney slices. Uterine metabolism is also shown in the latter solution.

Ringer phosphate glucose, falling off was noted from the 6-hour reading on, with the thyroxine offering some apparent protection from the start. In the presence of glycine, there was a slight difference at 6 hours, although the spread was not remarkable until 12 hours. In a similar experiment with alanine, no considerable difference between control and thyroxine vessels was seen until 24 hours, largely owing to the better general maintenance of tissue respiration in this medium.

Figure 4 also shows that uterus can be held within about 80 to 90 per cent of the starting metabolic rate with or without

DL-Alanine, certainly no better a buffer tl
shown outstanding effects (see Fig. 3). E
tissue metabolism was well maintained for
With added thyroxine, in the presence of gluc
sumption has increased as much as 30 per cent
with an average increase of 18 per cent in eig
In the three experiments charted in Figure 3,
per cent improvement in one day, and 15 p
The initial Q_{O_2} of the kidney tissue in D-alanine

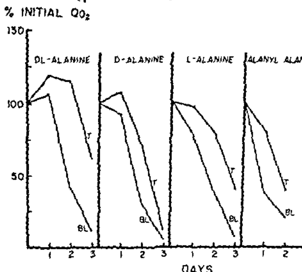


Fig. 3. Oxygen consumption of thyroidectomized ra
incubated in the various solutions shown. Metabolism
done at 37° C, incubation at 5° C without sha

higher than that in the DL-form which in turn
above that in the L. After 24 hours of incu
seemed somewhat superior to the L-alanine, bu
rapidly thereafter. Neither was comparable to
a discrepancy which deserves further study.

As with glycine, peptides of alanine, such as

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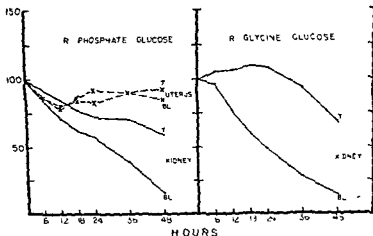


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Figure 4 also shows that uterus can be held within about 80 to 90 per cent of the starting metabolic rate with or without

thyroxine. This is one of the tissues showing no metabolic response to hypo- or hyperthyroidism in the experimental animal.

Before considering other amino acids, I should like to show the results with various substrates in Figure 5. With phosphate present, the "Mixed Substrates" (glycine, glutamate, pyruvate and fumarate) gave slightly better results than glucose for one day only, while glycine alone seemed inferior

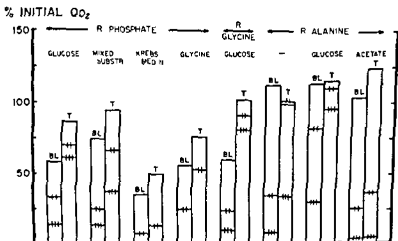


FIG. 5. Effects of various substrates on oxygen consumption of kidney slices. Each bar represents the percentage of initial Q_{O_2} at 24 hours (unadorned), 48 hours (2 vertical marks) and 72 hours (3 vertical marks).

as an energy source. The mixture proposed by Krebs (1950) as a superior medium ("Medium III", containing pyruvate, fumarate, glutamate as well as glucose) does elevate the starting oxygen consumption, but for maintenance was not as satisfactory with either phosphate or glycine as was glucose alone. Beyond the first 24 hours, acetate or pyruvate (the latter not shown in the figure) were not able to substitute for glucose in the presence of alanine, although the kidney slices performed remarkably well for two days with alanine without added material.

The results of survey experiments with a variety of amino

acids and related compounds are summarized in Table I. Serine behaved more like glycine than alanine, but threonine was nearly as effective as alanine. Both α- and β-amino*iso*-butyric acids, β-alanine and taurine showed remarkable maintenance properties with thyroxine present. Glutamine and glutamic acid in conjunction with thyroxine gave 18 and 12 per cent, respectively, better than starting respiration at

Table I

INCUBATION OF KIDNEY SLICES WITH THYROXINE IN THE PRESENCE OF VARIOUS AMINO ACIDS

Incubation Medium	*Start* Q_{O_2}		*Per cent of initial values after*					
			24 hr.		*48 hr*		*72 hr.*	
Ringer Glucose +	*BL*	*T*	*BL*	*T*	*BL*	*T*	*BL*	*T*
Glycine	3 15	3 20	37	89	13	72	7	67
Alanine	3 66	3 77	95	119	38	116	10	67
Serine	3 09	3 38	43	89	21	77	18	39
β-Alanine	3 24	3 95	54	107	24	90	10	75
Glutamic	3 29	3 49	42	112	15	59	6	26
Glutamine	3 55	3 27	59	118	24	83	9	26
Taurine	2 76	2 90	82	99	39	90		
α-amino-*n*-butyric	3 16	2 91	67	76	25	62		
Threonine	3 12	2 80	54	109	18	98		
α-Amino*iso*butyric	2 46	2 35	81	108	35	100	16	92
β-Amino*iso*butyric	2 60	2 50	68	103	32	97	8	87

24 hours, but fell off considerably thereafter. Except for the *N*-acetylalanine, already mentioned, none of these amino compounds has been as effective as alanine.

It is tempting to speculate about the possibility of formation of a thyroxylalanine or other peptide as the basis for the special type of activity seen here in the presence of amino acids. The diversity of structures found to be capable of active participation would seem to recommend a more thorough analysis before theorizing

Figures 6 and 7 show the results of incubation of kidney slices with different concentrations of several organic iodine

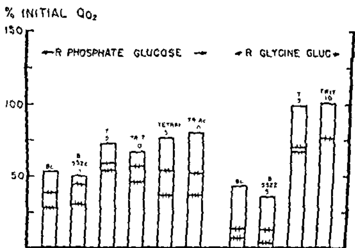


FIG. 6 Effects of various thyroxine analogues on oxygen consumption of kidney slices after incubation in Ringer phosphate glucose or Ringer glycine glucose Time indications as in Fig. 5

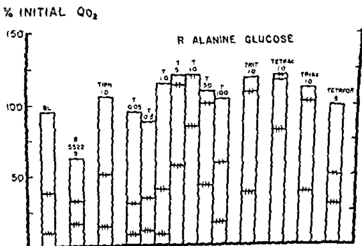


FIG. 7. Effects of various thyroxine analogues on oxygen consumption of kidney slices after incubation in Ringer alanine glucose Time indications as in Fig. 5.

compounds. Looking at thyroxine itself, slight effects can be seen with 1·0 μg. per ml., but the best results required 5–10μg. The 5 μg. level was used in most of the experiments being

Table II

SUMMARY OF THYROXINE EFFECT ON KIDNEY SLICES INCUBATED IN VARIOUS MEDIA

Incubation Medium		*Start* Q_{O_2}	*Per cent of initial values after*		
			24 hr.	*48 hr.*	*72 hr.*
		*BL T**	*BL T*	*BL T*	*BL T*
R Phosphate glucose	(8)†	3 11 2 90	57 79	31 64	21 56
R Glycine glucose	(7)	2 97 2 92	56 101	19 82	8 71
R Alanine glucose					
5 μg /ml thyroxine	(3)	3 80 3 86	93 122	36 114	9 57
10 μg /ml. thyroxine	(5)	3 62 3 68	104 117	48 118	17 88

* 5 μg L-thyroxine per ml incubation fluid except where shown with alanine.
† Number of experiments

reported here, but it appears from the summaries in Table II that much better maintenance resulted at three days when 10 μg. were used. Some falling off occurred when the levels were as high as 50–100 μg.

When tetraiodophenolphthalein (TIPH) was added to the Ringer alanine glucose system, there was no consistently better maintenance than with the blank. In the presence of *N*-(4-hydroxy-3 : 5-diiodobenzyl)-3 : 5-diiodotyrosine ("B-5522"), there actually appeared to be some decrease from the control tissue maintenance, especially with the alanine system. Both of these organic iodine compounds are without metabolism-stimulating properties when injected into normal or thyroidectomized animals. At higher levels, B-5522 has been shown to antagonize the metabolism-supporting action of thyroxine in athyreotic rats (Barker *et al.*, 1950).

One experiment is shown in Figure 4 with tetraiodothyroformic acid (TETRFOR), an analogue found in this laboratory to have about 4 per cent as much metabolism-stimulating action as L-thyroxine. It was only slightly better in these incubations than its own control figures, included in the averages for the "blanks". These results with TIPH, B-5522 and TETRFOR indicate a much higher biochemical specificity for the incubation procedure than for the *in vitro* effects on succinoxidase. However, TRIT was no more effective on a concentration basis than thyroxine, and the acetic acid analogues TETRAC and TRIAC were approximately as active. We feel that it is still too early in our analysis of this phenomenon to tell whether these quantitive discrepancies, particularly between thyroxine and TRIT, may furnish clues to mechanism of action. Many *in vitro* and *in vivo* differences have been considered, and one should not overlook differential rates of destruction, etc. The first possibility has already been examined *in vivo*; the only major difference we have encountered is a considerably greater effect of the triiodo compounds on heart metabolism (Barker, 1956), although Lerman and Pitt-Rivers (1955) reported a dissociation between metabolic and other clinical effects with TRIAC.

I should like to indicate some of our excursions to find out more about what is going on during this type of incubation. Paper chromatography of the medium after incubation has revealed the presence of small amounts of phenylalanine, leucine, glutamic acid, serine and lysine in addition to the added material. Probably originating in the tissue, these amino acids appear regardless of what one has added at the start of the experiment. The presence or absence of thyroxine does not appear to influence the variety or the quantity of amino acids liberated into the fluid.

Chromatography of methanol-ammonia extracts of centrifuged incubation medium and tissue indicates that added thyroxine was completely taken up by the tissue slices. This picking up of thyroxine appears to be incomplete after 75 minutes of incubation at 5° C, but is complete after 75 minutes

7°. At the end of a three-day incubation run, TETRAC consistently been demonstrated in the tissue and TRIT infrequently.

Summary

ices of kidney cortex from thyroidectomized rats have been bated in various solutions for as long as 3 days at 5° C. gen consumption has been measured at daily intervals at and values compared to pre-incubation runs.

ı Krebs' Ringer phosphate glucose solution, addition of oxine is able to decrease, but not prevent, the marked ease of respiration occurring over the 3 days. When glycine bstituted for the phosphate, the tissue can be maintained he starting metabolic rate for 24 hours with thyroxine ent.

ı Ringer alanine glucose, the tissue oxygen consumption ' be as high as 130 per cent of the initial value after 24 rs The average of eight experiments was 118 per cent at day and 115 per cent at two days Results are more able at three days, but our most recent runs have shown maintenance as high as 87 to 95 per cent of that at the t can be achieved.

s substrates, pyruvate, acetate, and mixtures of pyruvate, arate, glutamate and glucose have proved inferior to ose alone.

esides alanine, threonine, α-amino*iso*butyric acid, β-amino-utyric acid and β-alanine have given good results. Al-ugh not strictly comparable in structure, *N*-acetylalanine taurine have shown effects warranting further study.

'his reaction of excised tissue may throw some light on the roxine control of metabolism.

nowledgement

he author wishes to thank Messrs W. J. Lewis and R. H. Lindsay assistance with the work reported herein.

REFERENCES

BARKER, S. B. (1956). *Proc. Amer. Goiter Ass.* In press.
BARKER, S. B., KIELY, C. E., Jr., DIRKS, H. B., Jr., KLITGAARD, H. M., WANG, S. C., and WAWZONEK, S. (1950). *J. Pharmacol.*, 99, 202
BARKER, S. B., and LEWIS, W. J. (1956*a*). *Fed. Proc.*, 15, 8.
BARKER, S. B., and LEWIS, W. J (1956*b*). *Proc. Soc. exp. Biol., N.Y.*, 91, 650.
BRUICE, T. C., WINZLER, R. J., and KHARASCH, N. (1954). *J. biol. Chem.*, 210, 1
CLARKE, E C., and BALL, E (1955). *Fed. Proc.*, 14, 193
GEMMILL, C. L. (1952). *Amer. J. Physiol.*, 170, 502.
KREBS, H. A. (1950). *Biochim. biophys. Acta*, 4, 249.
LERMAN, J., and PITT-RIVERS, R (1955). *J. clin. Endocrinol. Metab*, 15, 653.
THIBAULT, O. (1955). *C.R. Soc. Biol., Paris*, 149, 877.
THIBAULT, O., and PITT-RIVERS, R (1955) *C.R Soc. Biol, Paris*, 149, 880.
WISWELL, J. G., ZIERLER, K. L, FASANO, M. B, and ASPER, S. P., Jr. (1954) *Johns Hoph. Hosp. Bull.*, 94, 94.

DISCUSSION

... estimated not with serum itself having known quantities of

glycine. ... chromatograms show that there is not more

... acid during the three days.

Barker: Apparently not when glucose is there. The substrates which we have tried so far do not include any dicarboxylic acids but do include pyruvate, lactate, acetate and glutamic; glucose gives us best maintenance by far.

Lardy: You had good maintenance with alanine without glucose. One of your slides showed alanine to be far superior to anything else in the absence of glucose.

Taurog: How did you identify the TETRAC that you claim to be present? And secondly, do you think that the action that you were getting was due to some transformation of the thyroxine or due to some other action of thyroxine such as the maintenance of enzyme levels, as you seemed to favour in your previous argument?

Barker: My prejudice, as stated before, is towards the maintenance of cellular integrity, or at least something approaching it I am just adopting that as an arguing point—as with insulin, the evidence is mostly indirect. To detect TETRAC we used methanol-ammonium acetate, butanol-ammonia, tertiary butanol-ammonia and tertiary amyl-ammonia compared simultaneously or with two-dimensional chroma-

thyronine and TETRAC?

Barker: Yes, except that ninhydrin is so much less sensitive than the ceric sulphate that we do not consider it really comparable

Wilkinson: But the point I was making was that this ceric sulphate reaction is given both by TETRAC and triiodothyronine.

Barker. We have depended more on an R_F separation in a variety of solvent mixtures because our experience with ninhydrin, even where you have an amino acid, is that it is so relatively insensitive that we would prefer to try to separate with a variety of solvents and then use the ceric sulphate reagent.

... I hope to compare notes with you We have used a few minor tricks—such as ...

Barker: We do not see it until after 12 to 24 hours. We especially hope to be able to set up a very reproducible system which some other

cids,
low-
say
mall
amount of free alanine from the *N*-acetyl form) α-amino*iso*butyric, β-amino*iso*butyric and threonine, when you add thyroxine you get a

I feel we are dealing here with

Barker: Yes.

Lardy: It would be nice if Prof. Barker could make some sort of extract of his preparation like Mme Thibault has done and see if he gets any effect.

Barker: That we did, we tried making just a high-speed centrifugation of the saline homogenates; we tried extracting with butanol or with methanol. In no case did we get any material which would produce an immediate effect. Dr. Lardy, maybe you and I are using the word 'synergistic' differently.

Lardy: Well, in order to show synergism you would have to give us

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ven
greater

ut will not

respiration

Lardy: from falling off and amino acids prevent it but the combination of the

ne and amino acid is not better than the additive effect of either think that any evidence for a unique peptide thyroid hormone

ne.

cent of the initial Qo_2.

ault. I wish first to congratulate Prof. Barker on his incubation nents. A very interesting fact to me was pointed out by Prof —that respiration of some tissues is not affected by hormones. t is the same tissues which are very easily sensitized, spleen,

er: No, I am afraid I cannot, because the addition of the acetic ialogue was no better than the thyroxine or triiodothyronine

ue potentiation of the epinephrine, but often nothing at all. This ething like the adenergic blocking effects being much greater on you have stimulatory responses to the epinephrine

adding or whether we are forcing so much amino acid material into the cell that cellular disintegration—whatever it is due to—goes on less

to maintain tissue for a day, now we are dissatisfied with 3 days and want to get up over 30 per cent stimulation.

Lardy: Is this an immediate stimulation?

Barker: We do not see it until after 12 to 24 hours. We especially hope to be able to set up a very reproducible system which some other

stimulatory effect with each agent so you would expect the two together to be better than either alone. Do you get anything over and above the additive effect of each alone?

Barker: Oh yes. We have several different groups of amino acids, some of which are no better than the Ringer phosphate glucose. However, with *N*-acetylalanine (for no reason I can suggest and I must say that paper chromatography shows liberation of only a very small amount of free alanine from the *N*-acetyl form) α-amino*iso*butyric, β-amino*iso*butyric and threonine, when you add thyroxine you get a clear and consistent effect over that. I feel we are dealing here with some aspect of amino acid or protein metabolism which thyroxine is able to alter favourably.

Lardy: Is the effect greater than the sum of what each would do alone?

Barker: Yes.

Lardy: It would be nice if Prof. Barker could make some sort of extract of his preparation like Mme Thibault has done and see if he gets any effect.

Barker: That we did; we tried making just a high-speed centrifugation of the saline homogenates; we tried extracting with butanol or with methanol. In no case did we get any material which would produce an immediate effect. Dr. Lardy, maybe you and I are using the word 'synergistic' differently.

Lardy: Well, in order to show synergism you would have to give us

greater. ... supports it in that case, does it?

but will not

ts respiration

from falling off and amino acids prevent it but the combination of the

the patient was sent out of hospital and treated with thyroxine. Comparison of the results suggested that of the two products TRIAC had the greater effect on blood cholesterol (Trotter, 1956). However, the fact that one was given in hospital, the other while the patient was at home, leads me to distrust the comparison, as differences of diet, etc., may have affected the blood cholesterol level.

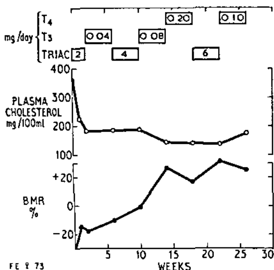


FIG. 1 Effect of TRIAC, triiodothyronine and thyroxine on plasma cholesterol and BMR in a case of myxoedema

I have greater confidence in the results obtained with the other two cases of myxoedema. Here the patients received either TRIAC, triiodothyronine (T_3), or thyroxine (T_4) for periods of two to four weeks. The patients were living at home but spent the last night of each experimental period in hospital in preparation for a BMR the next morning. The plan of the experiment is illustrated in Figure 1; the other case of myxoedema was treated in a very similar way.

Since the point of interest is the relative effect on blood cholesterol and BMR, the results are presented as a plot of

SOME OBSERVATIONS ON THE CLINICAL EFFECTS OF TRIIODOTHYROACETIC ACID

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THE observations which I propose to describe were instigated by Lerman and Pitt-Rivers' report (1955) on two cases of myxoedema treated with triiodothyroacetic acid (TRIAC). In both instances there was a prompt clinical response, a marked fall in blood cholesterol, but no change in basal metabolic rate (BMR). These observations made one wonder whether TRIAC was a form of thyroid hormone with a dissociated action, having a more marked effect on blood cholesterol than on BMR. We have long been familiar with this type of dissociation in the effects of the adrenocortical hormones; it did not seem impossible that a similar state of affairs might exist with the thyroid hormones. I have attempted in a tentative way to look into this possibility. The first cases studied seemed to support the suggestion, but later results suggest that there is no essential difference between the action of TRIAC and that of the other thyroid hormones. These studies are still incomplete and inconclusive, and I think their main interest is in provoking ideas on the effect of thyroid hormones on blood cholesterol and on BMR. I am far from clear, for instance, whether the effect on cholesterol levels is regarded as a by-product of the general stimulation of metabolism, or whether it is a specific action of the hormones.

I have tried to compare the relative effect of TRIAC and the other hormones on blood cholesterol and BMR in three of myxoedema. The first case I treated showed a prompt

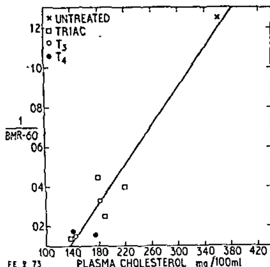


FIG. 2 The values for the BMR have been expressed in the conventional way, where average normal equals 100 per cent A figure of 60 per cent has been subtracted from all values and the reciprocal has then been plotted against plasma cholesterol Same case as in Fig 1.

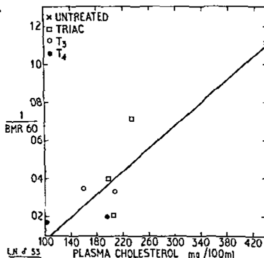


FIG 3. Another case of myxoedema. Results plotted in the same way as in Fig 2.

these two parameters (Figs. 2 and 3). In order to achieve approximate linearity the cholesterol level has been plotted against the reciprocal of the BMR, after deducting an arbitrary value of 60 per cent (or −40 per cent, where average normal is taken as 0 per cent) as representing the part of the BMR which is not under thyroid control. Figures 2 and 3 show that when the results in the two cases of myxoedema are plotted in this way the relative effects of the three substances appear to be much the same. These studies are being continued.

I then turned to euthyroid subjects and examined the ability of the three hormones to lower the blood cholesterol level, and also radioiodine uptake. The hormones were given for periods of two weeks with single estimations at the beginning and end of each period. The results are shown in Table I.

Table I

THE EFFECT OF DAILY ORAL DOSES FOR TWO WEEKS ON PLASMA CHOLESTEROL AND RADIOIODINE UPTAKE OF EUTHYROID SUBJECTS. RADIOIODINE UPTAKE WAS MEASURED AS THE RATIO OF NECK COUNT TO THIGH COUNT AT TWO HOURS AFTER A TRACER DOSE OF ^{131}I

Substance	*Dose (mg./day)*	*No. of Subjects*	*Mean initial cholesterol*	*% fall in cholesterol*	*Mean initial ^{131}I uptake*	*% fall in ^{131}I uptake*
Thyroxine	0 2	5	194	10	6·5	41
Thyroxine	0 4	5	231	29	7·5	69
Triiodothyronine	0 04	7	245	12	8·3	41
Triiodothyronine	0 08	5	234	21	7·5	68
TRIAC	1 0	3	210	14	8·0	61
TRIAC	2 0	6	235	33	8·9	73
TRIAC	3 0	5	222	20	5·1	54
TRIAC	4 0	5	214	23	6·7	81
Inert	—	6	210	1	5 3	0

It can be seen that all three hormones lowered the blood cholesterol, the maximal effect being about 20–30 per cent. Triiodothyronine was about five times as potent as thyroxine. The results with TRIAC were more variable, but it seems that the effect of 1 mg. was at least as great as that of 0·2 mg.

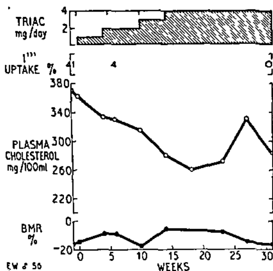


FIG. 5. Effect of TRIAC in an euthyroid subject with coronary artery disease.

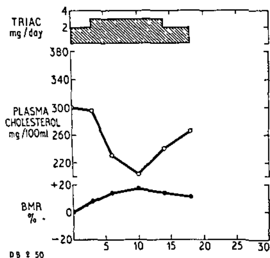


FIG. 6 Effect of TRIAC in an euthyroid subject who may have coronary artery disease.

thyroxine, while 2 mg. or more had about the same effect as 0·4 mg. thyroxine. It is therefore probable that TRIAC may have about a fifth of the potency of thyroxine. The relatively greater effect of TRIAC on radioiodine uptake may possibly be partly due to the larger amounts of iodide which may be released as the total dose is greater.

Four other euthyroid subjects have been studied in greater detail. They have been given gradually increasing doses of

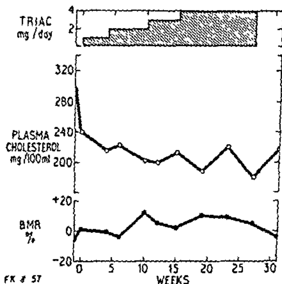


FIG. 4. Effect of TRIAC in an euthyroid subject with coronary artery disease.

TRIAC, up to 4 mg. daily for several months, and the effect on blood cholesterol and BMR has been observed at two- to four-week intervals. Three of these subjects had definite coronary disease, and the fourth may well have had it, though she has not had a definite infarct. The results are shown in Figures 4, 5 and 6 (the remaining case has been described in full elsewhere—Trotter, 1956). In three cases there was a definite fall in blood cholesterol, but in the remaining case (Fig. 4) the result was indefinite as the cholesterol level was falling at the time TRIAC was started, and has not yet

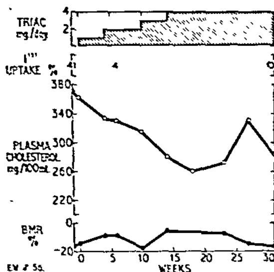


FIG. 5. Effect of TRIAC in an euthyroid subject with coronary artery disease.

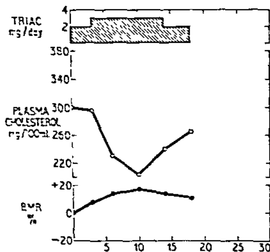


FIG. 6. Effect of TRIAC in an euthyroid subject who may have coronary artery disease.

returned to its original level after stopping the drug. In another case the marked fall in cholesterol level has not been entirely maintained by a dosage of 4 mg. daily (Fig. 5). In all four cases the BMR appears to have risen slightly, though it is not possible to be quite certain that a real change has occurred.

Inspection of these graphs suggests that an unexpectedly large fall in cholesterol has occurred in relation to the relatively trivial rise in BMR. This is demonstrated by plotting reciprocal BMR against blood cholesterol; an example is shown in Figure 7. It will be seen that the shift in BMR, for a given

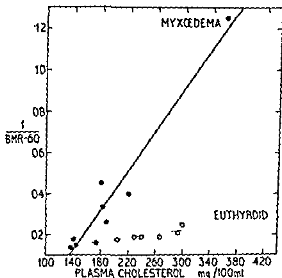


FIG. 7. Similar plot to Figs 2 and 3. The case of myxoedema is the one shown in Fig. 2; the euthyroid patient is the one shown in Fig. 6.

change in cholesterol level, is less in the euthyroid than in the myxoedematous subject. When the regression of BMR on ... that the slope is less in the

series the blood cholest...

to BMR in the euthyroid than in the myxoedematous subjects. This seems to me an unexpected and surprising result, and I am at a loss to explain it. In view of the results obtained with TRIAC, triiodothyronine and thyroxine in the two cases of myxoedema, I am reluctant to attribute it to a specific effect of TRIAC. The four subjects are now going to be treated with thyroxine, which should settle this point.

I wondered whether it was possible to attribute the relatively labile cholesterol to something peculiar to the subjects

Table II

THE SLOPE OF THE REGRESSION OF THE RECIPROCAL OF THE BMR (AFTER SUBTRACTION OF A BASAL VALUE OF 60 PER CENT) ON BLOOD CHOLESTEROL IN THREE CASES OF MYXOEDEMA AND IN FOUR EUTHYROID SUBJECTS WITH CORONARY ARTERY DISEASE

Sex	*Age*	*Diagnosis*	*Slope*	*Correlation Coefficient*
F	73	Myxoedema	0 00049	+ 0·86
M	53	Myxoedema	0 00030	+ 0 70
M	58	Myxoedema	0 00023	+ 0 13
M	72	Coronary artery disease	0 00008	+ 0 20
M	57	Coronary artery disease	0 00008	+ 0 23
M	56	Coronary artery disease	0 00008	+ 0·51
F	50	? Coronary artery disease	0 00006	+ 0 76

rather than to the drug. I have calculated the mean value for the slope of the regression line for several groups of patients, with and without thyroid disease, described in the literature (Table III). As these calculations had to be made from only a few points in each case (sometimes only two) there is naturally a very large individual scatter. The means for the three groups described by Gildea, Man and Peters (1939; Man, Gildea and Peters, 1940) do, however, agree quite closely with each other, and with Turner and Steiner's (1939) values for euthyroid subjects. Since the range of BMR and cholesterol values was very different in the thyrotoxic and myxoedematous groups,

this close agreement suggests that the method of plotting is not unreasonable.

I have also included values for the slope calculated from data given by Blumgart's group (Gilligan *et al.*, 1934) for cardiac patients treated by total thyroidectomy. The mean values are considerably higher than for any of the other series, possibly owing to differences of technique It is, however, clear that there is no difference between the congestive cardiac

Table III

MEAN VALUES FOR THE SLOPE OF THE REGRESSION OF THE RECIPROCAL OF THE BMR (AFTER SUBTRACTION OF A BASAL VALUE OF 60 PER CENT) ON BLOOD CHOLESTEROL IN THE PRESENT AND SOME REPORTED SERIES.

Author	*Diagnosis*	*No. of Cases*	*Mean Slope*
Present series	Myxoedema	3	0 00034
Present series	Euthyroid with coronary artery disease	4	0 00007
Gildea, Man and Peters (1939)	Myxoedema	14	0·00021
Gildea, Man and Peters (1939)	Euthyroid	6	0 00034
Man, Gildea and Peters (1940)	Thyrotoxicosis	21	0 00027
Turner and Steiner (1939)	Euthyroid	18	0 00023
Gilligan *et al.* (1934)	Congestive cardiac failure	17	0 00074
Gilligan *et al.* (1934)	Angina	9	0 00072

failure group (mainly consisting of patients with rheumatic heart disease) and the group with angina, who may be presumed to be suffering from coronary artery disease. I conclude from the absence of any difference between these two groups that it is unlikely that the low values for the slope in my four euthyroid subjects can be attributed to the fact that they have coronary artery disease.

I am therefore unable to explain why my four euthyroid patients show such relatively large changes in cholesterol level, when the corresponding BMR values have changed so little.

Summary

1. TRIAC has been given to three cases of myxoedema. In two of these the relative effect of TRIAC, triiodothyronine and thyroxine on blood cholesterol and BMR were much the same. In the third case TRIAC appeared to have a relatively greater effect on blood cholesterol, when compared with thyroxine.

2. TRIAC, triiodothyronine and thyroxine all lowered the blood cholesterol levels of euthyroid subjects, and also depressed the uptake of radioiodine. Triiodothyronine was about five times as potent as thyroxine in both respects. TRIAC appeared to have about one-fifth of the activity of thyroxine.

3. Four euthyroid patients, with coronary artery disease, were given TRIAC in gradually increasing doses for periods of several months. The blood cholesterol levels decreased in all the subjects. The BMR appeared to increase but the change was so slight that it may not be significant. The cause of this relative lability of the blood cholesterol is discussed. In view of the results obtained with the myxoedematous subjects it is difficult to attribute it to a specific effect of TRIAC. From other reports in the literature it seems unlikely to be an attribute of patients with coronary artery disease.

Acknowledgements

[illegible]

REFERENCES

GILDEA, E F., MAN, E B., and PETERS, J. P. (1939) *J. clin. Invest.*, **18**, 739.

GILLIGAN, D R, VOLK, M. C., DAVIS, D., and BLUMGART, H. L. (1934). *Arch. intern Med.*, **54**, 746.

LERMAN, J., and PITT-RIVERS, R. (1955) *J. clin. Endocrin*, **15**, 653.

MAN, E. B., GILDEA, E. F., and PETERS, J. P. (1940) *J clin. Invest*, **19**, 43.

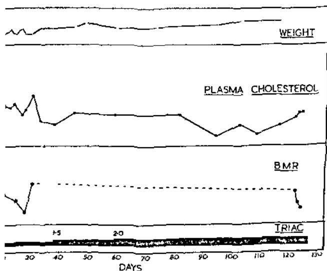
TROTTER, W. R. (1955). *Lancet*, **2**, 374.

TROTTER, W. R. (1956). *Lancet*, **1**, 885.

TURNER, K. B., and STEINER, A (1939) *J. clin. Invest.*, **18**, 45.

Ir. Jacob Lerman in Boston has demonstrated in four of
effect on the blood cholesterol and no effect whatsoever
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. 1 (Goolden). Effect of TRIAC in myxoedema.

A moderate degree of hypothyroidism induced by means

progress was judged clinically and by serial estimations of plasma

readmitted to hospital so that his thyroid status might be fully assessed BMR and plasma cholesterol estimations were made on three consecutive days. The mean value for plasma cholesterol was 235 mg. per cent and the mean of three BMR estimations −25 per cent. The absence of angina and increased exercise tolerance are perhaps a more reliable

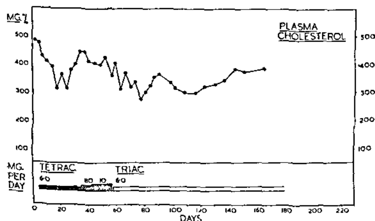


FIG 2 (Goolden) Effect of TETRAC and TRIAC in hypercholesterolaemia.

index of reduced basal metabolism than a BMR There is some evidence to suggest that in this patient a dose of 2 mg of TRIAC daily is sufficient to prevent any of the signs or symptoms of hypothyroidism and to maintain a normal plasma cholesterol level but not to restore the BMR

Figure 2 shows the effect of the acetic acid analogues of thyroxine and triiodothyronine on plasma cholesterol in a patient with familial

escape from this effect, which was not reversed when the dose of TETRAC

DISCUSSION

P.-Rivers: Dr. Jacob Lerman in Boston has demonstrated in four of his patients an effect on the blood cholesterol and no effect whatsoever on the BMR's. These patients received smaller doses of TRIAC than Dr. Trotter's; the average dose per day in the first two came to about 0 5 mg., and the other two to about 1·2 mg.; a weight loss was also observed.

If TRIAC is not different from thyroxine or triiodothyronine I should like some clinician to demonstrate this with triiodothyronine; with a small dose, say, 10 or 15 μg./day you might be able to show this differential effect on BMR and blood cholesterol. It is a very simple experiment for anybody who has access to myxoedematous subjects, and would settle this point.

Goolden: Acting on the suggestion that there might be a differential effect in cholesterol metabolism and BMR with TRIAC, the following two patients have been treated with this substance.

The first patient (Fig. 1) had for several years suffered from increasingly

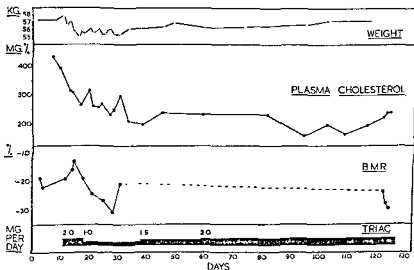


FIG. 1 (Goolden). Effect of TRIAC in myxoedema

severe angina A moderate degree of hypothyroidism induced by means

was started on TRIAC 2 mg. daily, which after a few days [illegible] reduced to 1 mg. daily because of slight angina and tachycardia. His

was not a proportionate increase in the BMR, but this may well have been a normal level for a patient of this age.

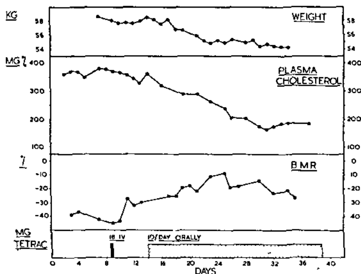


FIG 4 (Goolden). Effect of TETRAC in myxoedema.

On the results that have so far been obtained with TETRAC in

…remained low but his BMR went back to the original low level After he had been off TRIAC for a week, he started

was increased to 10 mg. daily. At this stage TRIAC in a dose of 6 mg. daily was substituted for TETRAC. The changes in the plasma cholesterol level on TRIAC were similar to those observed with TETRAC with a tendency to revert to the pre-treatment level. Neither substance had any side effects in the dosage used.

In a previous study a patient with myxoedema was treated with TETRAC (Goolden, A. W. G. (1956). *Lancet*, 1, 890) A clinical remission was obtained and the plasma cholesterol level and BMR reverted to normal on a dose of 8 mg daily

Figure 3 shows the response of a second myxoedematous patient

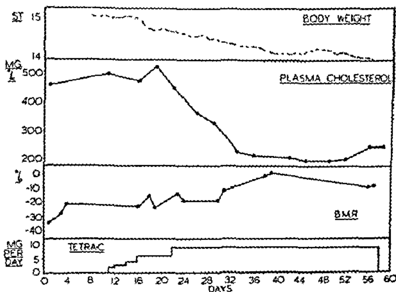


FIG. 3 (Goolden). Effect of TETRAC on body weight, plasma cholesterol and BMR in myxoedema.

to TETRAC. The pattern of response was similar to that of the first patient and she became euthyroid on a dose of 10 mg. of TETRAC daily, confirming that the potency of TETRAC in myxoedema is about one-thirtieth that of L-thyroxine

Figure 4 shows the effect of a single intravenous dose of TETRAC in myxoedema and was designed to study the latent period of action of this substance. After an intravenous dose of 18 mg. of TETRAC there was no appreciable effect on the signs or symptoms of myxoedema. An increase in the metabolic rate was not observed until 60 hours after the dose. The effect on plasma cholesterol was insignificant. The effect of a single dose was observed for a period of six days and the patient was then put on a dose of 10 mg. daily, on which he became euthyroid. Although the plasma cholesterol returned to within normal limits there

returned to the original levels within 6 days of stopping TRIAC. Considering the mean figures, there was no difference at all between the BMR's which were carried out at the beginning and end of the experiment, but two of the individual patients had rises of + 17 and + 20

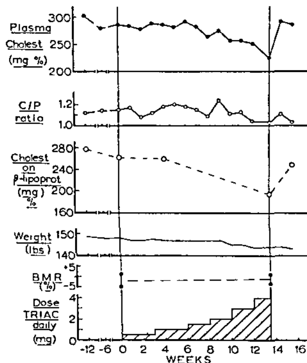


FIG. 2 (Macgregor) The effect of TRIAC on six euthyroid men with coronary artery disease (mean values).

cally myxoedematous

improvement was most dramatic, and after only 24 hours his whole appearance and mental attitude had altered.

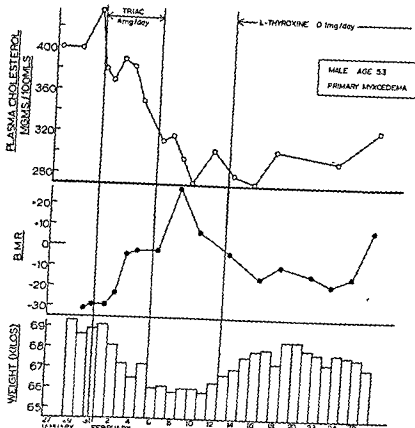


FIG. 1 (Macgregor). The effect of TRIAC on a patient with primary myxoedema.

carried in the β-lipoprotein fraction. Both it and the total cholesterol

THE INFLUENCE OF THE THYROID GLAND UPON IMMUNE RESPONSES OF DIFFERENT SPECIES TO BACTERIAL INFECTION

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The sole justification for an immunologist's presence at this symposium is that his complex, even esoteric, techniques have yielded facts that may illuminate a wider field of thyroid physiology than his own. I believe that the bacteriologist's

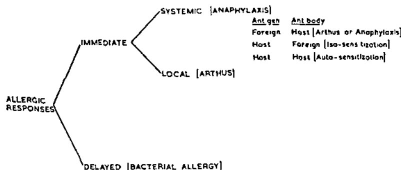


Fig 1. Allergic responses.

microscope need not necessarily restrict his vision, that he should think not only in terms of infection and immunity, but also in terms of their relationship with the non-specific metabolic response of the host to infection (Long, 1956), even though he risks leaving the limits of his own immunological experience and entering fields of study of which his knowledge is slight. If this should happen on this occasion, there are experts enough to lead me back to my own intellectual suburb

Bacterial infection may induce allergic and non-allergic

of the heparin production in the body under the influence of these drugs.

Macgregor: We were chiefly interested in this problem because Oliver and Boyd have been studying the effect of oestrogens on the total cholesterol and on the fraction of cholesterol carried on the β-lipoproteins. We did not get, in these patients receiving TRIAC, the same decrease in the amount on the β-lipoproteins with TRIAC which they had previously observed to follow the administration of ethinyloestradiol, though there was an overall fall in total cholesterol.

Trotter: Can you say if it was a fall in the α-lipoprotein?

Macgregor: No, there was not There was an overall fall of 20 per cent in the total cholesterol and of 27 per cent on the β-lipoprotein cholesterol. There was an absolute increase in the α-lipoprotein fraction.

Trotter: I seem to remember Oliver and Boyd saying that in a previous experiment with thyroxine they had found an equal fall in both α- and β-lipoproteins.

Macgregor: Yes. This result is really very much the same. There is not quite the same difference in the behaviour of the α- and β-lipoprotein fraction with either TRIAC or thyroxine as there is with oestrogens.

Barker: Could I request our Chairman to give her opinion as to whether there really is a difference in response to TRIAC and TETRAC?

P.-Rivers: Yes, TETRAC does not show so clearly the differential effect on BMR and blood cholesterol.

Barker: We have tried TETRAC with one myxoedematous patient, starting at 0·5 mg doses by mouth which were too small amounts to raise the BMR, and there was no change in the cholesterol. Increasing the dose to 3 mg. per day seemed to affect both BMR and cholesterol.

P.-Rivers. In Lerman's four patients the effect is one which you have not yet seen with thyroxine and triiodothyronine—four horizontal lines for the BMR's but a considerable fall in the blood cholesterols.

Macgregor. We are at present studying a myxoedematous patient to whom we have given 0 5 mg. doses of TRIAC daily So far it looks as though a cholesterol fall can occur at that dose level without a BMR rise. Her total cholesterol fell from 510 mg. per cent to 331 mg. per cent without any clinical improvement or rise in BMR.

P.-Rivers: That is the same dose as two of Dr Lerman's patients received?

Macgregor: Yes.

Table II
COMPARISON OF SPECIES

Species	*Tuberculin sensitivity*	*Ascorbic Acid Synthesis*	*Pharmacological Response to Cortisone*
Man Monkey Guinea pig	Readily induced	Ascorbic acid not synthesized	Resistant (antitoxin synthesis not depressed)
Rat Mouse Rabbit Ferret	Not readily induced	Ascorbic acid synthesized	Sensitive (antitoxin synthesis markedly depressed)*

* Antitoxin effect not known for ferret

Sodium thyroxine and anaphylaxis (immediate allergic response) in guinea pigs

A single injection of sodium thyroxine, 0·2 mg./kg., into guinea pigs converted a mildly-shocking dose of horse serum into a 100 per cent rapidly lethal dose. This effect is probably due to thyroxine-induced increased sensitivity to histamine phosphate (Fig. 2) The effect is not enhanced by giving

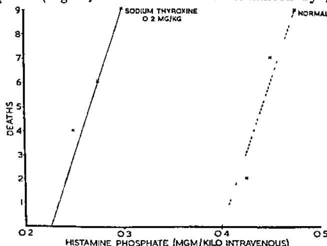


FIG. 2 Effect of sodium thyroxine on toxicity of histamine phosphate for guinea pigs.

responses in susceptible species. The allergic responses can be divided into immediate and delayed types (Fig. 1). Anaphylaxis provides an example of the former and the tuberculin response of the latter. The essential differences between the two groups are tabulated (Table I).

Table I

Comparison of Anaphylaxis and Bacterial Allergy

*Anaphylaxis**	*Bacterial Allergy (Tuberculin type)*
Laboratory phenomenon	Natural phenomenon
Sensitivity induced by a single injection of allergen	Sensitivity induced in three weeks by chronic low grade infection
Response immediate	Response delayed
Circulating antibody	Fixed antibody
The sensitized state can be passively transferred with serum	The sensitized state can be passively transferred with cells
Histamine has a major rôle in inducing symptoms	Histamine has a minor rôle in inducing symptoms.
Antihistamines partly relieve symptoms	Antihistamines do not relieve symptoms
Cortisone does not relieve symptoms	Cortisone relieves symptoms
The characteristic lesion is spasm of smooth muscle	The characteristic lesion is the inflammatory necrotizing response

* The Arthus phenomenon can be considered to be a type of "local anaphylaxis" affecting the smooth muscle of blood vessels

The ability to become "allergic" differs with species. The delayed (tuberculin-type) hypersensitivity is readily induced only in man, monkey and guinea pig. These species have in common two further characteristics—inability to synthesize ascorbic acid and resistance to the toxic actions of cortisone—both of which are intimately concerned with the tuberculin response (Long, 1954, 1955, 1956) (Table II). The resistance of different species to the toxic actions of cortisone, namely the loss of weight and depression of plasma γ-globulin and antitoxin levels, has been fully described by Shewell (1955) and summarized by Shewell and Long (1956). This division into two groups (Table II) is important when we come to consider the response of these species to thyroxine.

results and reached similar conclusions. The analogy between susceptibility to experimentally-induced diabetes and sensitivity to tuberculin is a strong one (Long, 1954, 1955, 1956).

Houssay (1950) and his colleagues (1946) have studied experimental diabetes mellitus. My colleagues and I have worked on desensitization to tuberculin (Long and Miles, 1950; Long, Miles and Perry, 1951*a*, *b*; Long and Shewell, 1954). Under comparable conditions, thiouracil and thyroxine, that is to say hypo- or hyperthyroidism, oppose both the experimental production of diabetes mellitus and of desensitization.

Houssay (1950) attributes the beneficial anti-diabetogenic effect of hypothyroidism to an increase in the free —SH groups of the tissue, and that of hyperthyroidism to an increase in islet tissue associated with hyperinsulinism (Houssay, Foglia and Martinez, 1946). As already pointed out, such an hypothesis will equally well explain the relationship of the thyroid gland to tuberculin sensitivity.

The remarkable analogy between the biological activity of cortisone and of alloxan (Long, Miles and Perry, 1951*a*, *b*; Long, 1955) supports the suggestion that —SH metabolism is intimately concerned with sensitivity to tuberculin and with the action of the thyroid gland upon it.

Sodium thyroxine and antitoxin production in guinea pigs

There is a close analogy between the influence of the thyroid gland on sensitivity to tuberculin and its influence on synthesis of diphtheria antitoxin Preliminary experiments showed that treatment of guinea pigs with thyroxine greatly increased both the level of circulating antitoxin and associated immunity to intradermally-injected diphtheria toxin. Indeed, the latter provided a measure of the former in thyrotoxic, as in non-thyrotoxic guinea pigs (Hartley, 1934; Miles, 1949; Long, 1950*) The increase in immune response was greatest

* Three references to immunological techniques.

thyroxine over a long period of time; it may therefore be due to a direct action of thyroxine upon the host (cf. the effect of thyroxine on sensitivity to tuberculin and on antitoxin production (see below)). Comparable results have been obtained, in thyrotoxic guinea pigs, with the Arthus phenomenon ("local anaphylaxis"), and with histamine liberators injected both locally and intravenously (Long, unpublished).

Sodium thyroxine and sensitivity to tuberculin (delayed allergic response) in guinea pigs

Using the response to intradermal tuberculin as a measure of allergic hypersensitivity in albino guinea pigs sensitized with BCG, it was found that sodium thyroxine increased sensitivity (Long and Miles, 1950; Long, Miles and Perry, 1951*a*, *b*). The effect was enhanced by prolonged treatment with a dose of thyroxine insufficient to cause a loss of weight. A single injection of thyrotrophic hormone, of sodium thyroxine, of triiodothyronine or of triiodothyroacetic acid did not increase sensitivity significantly These facts suggest that the action of the thyroid gland on the tuberculin response is *indirect* (cf. the effect of thyroxine on immediate allergic responses).

Further work showed that insulin increased sensitivity to tuberculin (Cornforth and Long, 1953); partial pancreatectomy had no effect on sensitivity but prevents the action of thyroxine in increasing sensitivity. However, partial pancreatectomy does not prevent the increased response to tuberculin that follows the injection of insulin. It was concluded that thyroxine increases sensitivity to tuberculin in guinea pigs by increasing the amount of islet tissue and so inducing hyperinsulinism (Long and Shewell, 1954).

One of the criticisms that has been made of this work is that it is based on the behaviour of a single tissue, namely the response of the skin to either toxins or allergens. This criticism carries little weight, for Houssay (1950), using widely different techniques, has obtained remarkably similar

The relationship of endocrine activity to non-protein —SH and ascorbic acid metabolism (Long, 1955) is also being actively studied by my colleagues and myself. Time will show whether these clues are as important as we believe them to be, and whether the exploitation of a single tissue with a simple technique was worthwhile

REFERENCES

CORNFORTH, J. W., and LONG, D. A. (1953). *Lancet*, **1**, 160.
HARTLEY, P. (1931) *Wiss Woche Frankfurt a M.*, **3**, 81.
HOUSSAY, B. A. (1930). *Amer. J med. Sci.*, **219**, 353.
HOUSSAY, B. A., FOGLIA, V. G., and MARTINEZ, C. (1946). *Endocrinology*, **39**, 361.
LONG, D. A. (1950). *Brit J. exp. Path.*, **31**, 183
LONG, D. A. (1954). *Lancet*, **1**, 529.
LONG, D A. (1955) *Int. Arch Allergy*, **6**, 337.
LONG, D A. (1956) *Canad. med. J.*, **74**, 771.
LONG, D. A., and MILES, A. A (1950) *Lancet*, **1**, 492.
LONG, D. A., MILES, A. A, and PERRY, W L M. (1951*a*). *Lancet*, **1**, 1392.
LONG, D A., MILES, A A., and PERRY, W L M. (1951*b*) *Lancet*, **2**, 902.
LONG, D A., and SHEWELL, J. (1954) *Brit. J. exp Path*, **35**, 503.
LONG, D. A., and SHEWELL, J. (1955) *Brit. J. exp Path*, **36**, 351
MILES, A A. (1949). *Brit. J. exp Path*, **30**, 319.
SHEWELL, J. (1955). University of London, Ph D. Thesis.
SHEWELL, J., and LONG, D. A. (1956). *J Hyg, Camb* In press.

DISCUSSION

C. N. H. Long: I do not quite understand your statement that the

steroid.

D. A Long: I defined cortisone-resistance in terms of loss of body weight and depression of γ-globulin synthesis. Resistance is a relative term. In cortisone-resistant species, body weight, nitrogen excretion

when treatment with thyroxine was prolonged and the dose repeatedly adjusted so that the animals continuously gained a little weight. An association was noted between a high level of circulating antitoxin and hypertrophy of both adrenal cortex and the islets of Langerhans. Hyperimmunity was prevented by partial pancreatectomy; it is almost certainly due to hypertrophy of the islets (Long and Shewell, 1955).

The nature of this effect of thyroxine upon antitoxic immunity in guinea pigs has been investigated by a new technique (Long, 1956) *designed to identify and measure the* different stages of immunity. Thyroxine speeds up the whole process of the development of antitoxic immunity in the guinea pig, and, in addition, increases the maximum immune response of which each animal is capable (Long, unpublished).

It may be significant, in view of the contribution of Prof. Vannotti, that guinea pigs with cirrhotic livers behave in a similar fashion (Long, 1956).

Extension of this work to other species showed that, in direct contrast to these effects in guinea pigs, thyroxine decreased and propylthiouracil increased the immune response of rats, mice and rabbits (Long and Shewell, unpublished). *One hypothesis being investigated is that this effect of thyrox-*ine is due to thyroxine-induced adrenocortical hypertrophy in "cortisone-sensitive" species (Table II).

Discussion

The division into cortisone-sensitive and -resistant species (Table II) is important in the study of thyroid activity. The fact that thyroxine causes hypertrophy of the adrenal cortex (sympathetic) and of the islets (parasympathetic), the actions of which are frequently antagonistic, is noteworthy and relevant to the species difference, for adrenocortical hyperactivity has a more profound effect in cortisone-sensitive than in *cortisone-resistant species.* A study of the wider implications of this phenomenon provides one of our current research themes.

D. A. Long: In both cases, the species were given as much food as they could eat, and the food intake was measured, and there was not actually an appreciable increase in appetite.

Querido: In rats there is no increase in intake under cortisone. We have done this with both ACTH and cortisone in carefully controlled studies. I, therefore, had the simple explanation that the increase in weight in human beings was more or less a question of appetite because they feel better and then eat more. So it seems not to be a specific effect

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decrease in these values

Zizine. I wonder if it is justified to say that adrenal hypertrophy

thyrotoxicosis does not seem to induce an increase of adrenal activity. In fact, several investigators have shown that thyrotoxicosis is often followed by a state of hypoadrenalism. As far as the question of thyroxine and sensitivity is concerned, we have seen several patients who were, at the same time, allergic and thyrotoxic; a cure of their thyrotoxicosis alleviated their allergic symptoms On the other hand, we have had myxoedematous patients who were at the same time allergic; the alleviation of their hypothyroidism was followed by a cure of their allergic condition

D. A Long: Is there not a distinction in thyrotoxicosis? I thought the point about thyrotoxicosis was that the adrenal is not active, and that there is a disbalance between the two, but in normal people adrenal activity varies with thyroid activity.

Zizine There may be a question of time between the onset of thyrotoxicosis and the adrenal activity

D A Long We certainly have not measured steroid output and our evidence of adrenocortical hypertrophy was based on increase in adrenal size and in the width of the adrenal cortex

Fraser. Dr. J Corvilain (1953 *Brit. med. J*, ii, 915) and I have made some observations on adrenal function in thyrotoxicosis. Looking at urinary 17-ketosteroid excretion, let us consider first the simpler

other evidence that they had received excess of the hormone and were reacting to it.

D. A. Long. I agree, but I limited it to those two points.

The effect of cortisone on resistance of different species to bacterial infection provides another example of differing resistance to an effect of cortisone. Bacterial infection resulting from the intradermal injection of organisms into guinea pigs or monkeys was not significantly affected by cortisone therapy. But the same organisms caused septicaemia and death in cortisone-treated rabbits with histological evidence of a failure in the cellular response to infection

The marked contrast in the effect of cortisone on the progress of tuberculosis in mice on the one hand and guinea pigs on the other provides another example of differing resistance

In all these respects man, monkey and guinea pig are analogous. Do you consider these reasonable examples of a distinction of resistance?

C. N. H. Long I am not certain that I am competent to answer that question. In your terms, yes, there would seem to be a difference. I am objecting to the general statement that there are species that are resistant Now, it may be so in this particular area, and as I say, you can show these differences. But this is not reflected in other aspects of cortisone overdosage

In the case of the guinea pig it has been shown that cortisone will produce hypertrophy and hydropic degeneration of the islets of Langerhans.

It is also interesting what you say about thyroid diabetes, and here again it may be that it is a matter of conditions under which this is examined Certainly Houssay showed first, and in some of our studies we also showed, that in a total diabetes removal of the thyroid had very little effect indeed

I think Dr. Barker has also done this. In the partially depancreatized animal some attenuation of the diabetes may occur, but I have always thought that this was a consequence of the drop in metabolic rate.

D A. Long Houssay and I were both working on the partially depancreatized animal.

C. N. H. Long: Yes, but the differences produced by thyroidectomy are not really very marked in comparison to those that follow removal of the hypophysis. I wondered in this study here why you did not remove the hypophysis, where you would have depressed the functional level of several endocrine organs.

To sum it all up, I am not at all sure, except in the limited sense that you are using it, that there is this marked species difference in the reaction to cortisone.

D A. Long: You are satisfied on body weights and on γ-globulin?

C. N. H. Long: Well, I am not even satisfied on body weight, because the administration of those enormous amounts of cortisone does odd things as you move from one type of patient to another, depending on how much water retention you get, how long it was given, and so on It is very hard to say merely on body weight.

Gross: I wonder if we could get an expression of opinion in this discussion as to the sites of action of the thyroid hormone in this particular context. Is this an effect directly on the adrenal; is it an effect on protein metabolism apart from species difference?

D. A. Long: I think all one can say is that the thyroid hormone has one effect on antibody in one group, and the reverse effect on antibody in the other group. I do not think the species difference in response can be ignored

in cortisone-sensitive species, but our present hypothesis is that it may be a consequence of adrenal cortex stimulation in these species.

P.-Rivers: Dr. Long, you said something about thyroxine, triiodothyronine and TRIAC all being equally ineffective in producing an immediate response, but I have an idea that you have done an experiment in which TRIAC had a remarkably rapid response.

D. A. Long: Yes, that is true. What the Chairman and I tried to do was to produce diabetes with a single injection of a very active thyroid hormone, and we tried the effect of TRIAC which we hoped would produce desensitization, which we would attribute to 'thyroid-diabetes'.

D. A. Long: It did not alter the tuberculin response.

slightly lowered. Thus, in myxoedematous subjects acute raising of thyroid dosage raised adrenal function, and treatment of thyrotoxic patients was followed by signs of lowered adrenal function. To this we must add that untreated thyrotoxicosis shows low urinary 17-ketosteroids, and, as noted, lowering of the thyrotoxicosis by treatment also lowers this further

D. A. Long: Thyrotoxicosis is a special case. I think myxoedema is more interesting.

Harris: But doesn't thiouracil have the same action in the normal animal? Several workers have found that administration of thiouracil causes adrenal atrophy in the normal animal.

Fraser: Yes, we have no data on that but I believe it is so.

Taurog: You mean that it is a direct effect of the thiouracil on the adrenal, or is the effect through the thyroid?

Harris: It doesn't appear to be through the adrenal gland since it has been shown (Zarrow, M. X., and Zarrow, I. G. (1951). *Proc. Soc. exp. Biol., N.Y.*, **76**, 620) that the atrophic adrenal gland of the thiouracil-treated animal still retains its sensitivity to exogenous ACTH.

Lardy: It might be on ascorbic acid synthesis (cf. 1948. *Arch. Biochem.*, **19**, 246). Did you use propylthiouracil in any of your work?

D. A. Long: Yes. Actually we cannot detect any difference between propylthiouracil and thiouracil although it has been suggested that the non-protein SH is higher if thiouracil is used.

Querido: Did you say that in thyrotoxics there was less than average normal ketosteroid output, and that they go up under thiouracil treatment?

Fraser: Gets lower still, acutely

Querido: And in the course of time?

Fraser: I am afraid we have not followed it a long time. Presumably in the long term, if you wait long enough, they will go back to normal.

Querido: The reason why I raise that point is because it seems difficult to conclude from ketosteroid excretion, if you are not talking about really low values, how much there is coming from the adrenals. A change in output does not necessarily mean a change in production. I mean, alteration of utilization in the periphery or some other factor may create a wrong impression.

Fraser: I quite agree. That is why I thought the more valid evidence of the relation was on the myxoedematous subject, and on that subject, the more you increase thyroid the more evidence there is of increased adrenal function. The changes following drug treatment of a thyrotoxic patient might be due to direct effects on either gland.

Querido: There probably it could work through the hypophysis. You have a very complicated preparation to put up the quantitative function, of which the parameters are far away.

Fraser: The myxoedematous patients were assessed on two different levels of thyroid maintenance dosage, on their standard dose and on double this dosage; thus a myxoedematous pituitary would not be involved in either case.

anterior pituitary control is totally inadequate to account for the

expose further problems I need say nothing to emphasize the deepness of our perplexity concerning the mode of action of thyroid hormone, a perplexity that persists in spite of the work that has been done on effects on isolated enzyme systems, and that was so impressively exemplified by Dr. Lardy in his contribution

Where then are we going, and are we following the best path towards our end? We are trying to do three things; first to identify the compound that acts in the body to produce the effects which we associate with the thyroid, if indeed there is a single substance that fulfils this rôle; secondly to understand the mechanisms that regulate the output of this substance, or perhaps of its precursor, from the thyroid; thirdly to unravel the precise mode of action of the active compound or compounds at the cellular level.

think that most of us feel any confidence that we know which of these compounds is the one that acts in the cell. We are discovering new physiological mechanisms in the control of thyroid function, but each of these discoveries pushes the ultimate control further back into the central nervous system, and at the same time reminds us of the complexity of the relationships between the different endocrine organs; we may well deceive ourselves and make things more difficult if we are in too much of a hurry to explain each new link in the total mechanism in simple terms, I need only remind you of the discussion of Prof. Harris's paper to emphasize this point. As to thyroid action at the cellular level, we have an unequivocal proof from the work of Dr Lardy and of others that thyroxine and compounds related to it can influence biochemical processes in separated sub-cellular particles in the direction that

GENERAL DISCUSSION

Harington At a recent Ciba Foundation conference which I attended the task that I have to undertake today fell to Sir Macfarlane Burnet, and he began by saying that he had been given the toughest assignment of the whole symposium. If he could say this, as he did with obvious sincerity although he is an active leader in the field that had been under discussion, what can be said of my position today? For the past three days I have listened with intense interest to the lively accounts and discussion of work that is going on in the field that we have been surveying; from a personal point of view the experience has been a chastening one, for it has inevitably reminded me forcibly how much of a back number I have become in thyroid research. On the other hand there is some satisfaction in the reflection that the great structure of the current physiological and biochemical work on the thyroid has been built in large part on the elementary facts concerning the chemistry and biochemistry of thyroxine that were discovered twenty to thirty years ago; one is indeed filled with a sense of something like awe when one sees the blossoming that can come from what in retrospect seem to have been a few simple and crude observations, when these are developed by later workers with more enlightened approach and more highly developed skills

The discussions that have taken place here during the past three days have ranged so widely and have covered the field so thoroughly that there is little indeed that I can usefully add; it would certainly serve no purpose for me to take up any point of detail, nor can I attempt to summarize effectively; all that I should like to offer are some general comments and suggestions; these arise from a consideration of the colloquium as a whole, I shall only refer specifically to those communications that are specially relevant to my theme

The blossoming of research on the thyroid that has taken place during the last ten years or so has of course led to an enormous increase of knowledge. As I have listened to the papers and discussions, however, I have realized more and more that such an increase in knowledge, encouraging as it is, may for the time being have the effect of making the ultimate attainment of our common goal more than ever remote. The complexity of the situation grows with the new facts that are revealed; even so far as the nature of the thyroid hormone itself is concerned we are forced

essential discovery is going to be made that will resolve our perplexities; it would be a pity if the true value of such a discovery were not recognized as soon as it should be because our eyes were too closely fixed on the minutiae that tend to absorb our interest.

P.-Rivers: Dr. Honor Fell is continuing tissue culture experiments with thyroid hormones but only on bone growth.

Barker: The tissues that respond favourably to thyroxine, triiodothyronine, TETRAC, etc., such as liver, kidney, and heart which we have been particularly interested in, are all structures that fail very rapidly. To my knowledge, nobody has been able

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Pulvertaft we have been examining the tissue culture of human thyroid material removed at thyroidectomy. Prof. Pulvertaft has found that he can keep it in a viable state for a week or more, but we have not really got very far on the biochemical studies yet and it would be rather premature to discuss any results

Lardy. One of my former colleagues, Dr. William Rutter who is now at the University of Illinois, is undertaking an extensive study of the effect of hormones on tissue cultures and has already obtained some interesting results with adrenocortical hormones. He has even planned some work with the thyroid hormone.

Taurog. I have a slide from another department which I thought I would throw into the discussion just to point out how complicated oxygen consumption is, and the interpretation of oxygen consumption measurements This is some work which was done at our University in the Institute of Experimental Biology on the effect of hydrocortisone on oxygen consumption of thyroidectomized rats I think it is quite an interesting finding.

Metabolic rates, in calories per sq. metre per hour, based on oxygen consumption measurements, are plotted against time in

twenty days after removal of the thyroids After about four

sumption up to and above the control values, and this in the absence

have done more hitherto than to suggest one or two means by which the thyroid hormone may stimulate cellular metabolism; the relation of the observed phenomena to thyroid action in the whole organism remains obscure. In all these fields, therefore, we are still far from the synthesis of knowledge that will be necessary before we achieve the final solution of our problems.

Now I should be most regretful if my remarks were taken to imply any under-valuation of the work that has been done and that is proceeding so actively in your hands. On the contrary, I have the greatest admiration for this work, and nobody is more anxious than I am myself to know the answers to some of the points that have come up in our discussions; for instance, the identification of the substance that Dr. Gross believes to be the transport form of triiodothyronine, and the synthesis of hydroxytriiodothyronine, followed by a study of its biological properties, are problems that are crying out for solution, and certainly ought to be pursued with vigour.

All I am pleading for is that the very rapidity with which new discoveries are forthcoming should not be allowed to cause too much preoccupation with detail, and that in our attempts to pinpoint the action of the thyroid hormone on individual biochemical processes we do not forget the organism as a whole. The point that I am trying to make is well taken in a letter that recently appeared in *Nature* from Sir Rudolph Peters entitled "Hormones and the Cytoskeleton", and which I think might be read with profit by anyone engaged in a field of research such as we have been discussing. In this letter Peters points out the errors that may arise if the biochemist allows himself, as he may be tempted to do, to regard the cell as a bag of enzymes and to forget that it has an organized structure, modification of one part of which will necessarily affect the whole. This consideration seems to me to be particularly relevant to the study of the action of thyroid hormones at the cellular and sub-cellular levels, and its extension to cover the whole of work on the physiology and biochemistry of the thyroid is surely sufficiently obvious. The thyroid gland after all, in itself and in its relations with other endocrine organs, exercises the most widespread and complex effects upon the body, and unless we constantly remind ourselves of this we shall not see the wood for the trees.

What I would suggest, therefore, is not that we should cease to accumulate new facts; this process must obviously go on. When a new fact is discovered, however, let it be thoroughly considered in the broadest possible context. Sometime or other, in each of the sections of the general field that we have been discussing, the

Taurog: I do not know if their nutrition was controlled.

Barker: Do you know if the adrenalectomized animals were

P.-Rivers: It is a short-term restoration; could you maintain your hypophysectomized or your thyroidectomized animals indefinitely on cortisone in the absence of thyroid?

Taurog: It was not so short—it was over a period of more than 25 days.

P.-Rivers: But could you have kept it up for six months?

Taurog: I do not know, but I think it is of interest to speculate at what sites these various hormones are acting to promote this increase of oxygen.

Macgregor. It does not need to be a hormone. You can raise the oxygen consumption of a myxoedematous patient by giving him simply large doses of aspirin.

Fraser: Or dinitrophenol.

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P.-Rivers. I should like to say how very much I have enjoyed this colloquium It has certainly provided much food for thought and although I feel that I am not much nearer knowing what the thyroid hormone is or does, at least it has given us some stimulus towards new experiments

of other hormones. I point this out to indicate how important other hormones may be in controlling oxygen consumption. Metabolic rate itself is a very complicated process, and I think we all recognize by now that the thyroid hormone is not the only one important in the control of metabolic rate.

C. N. H. Long: You do not get that effect in the intact rat with cortisone, do you? You do not get a rise in metabolic rate to the same extent in the intact rat, whereas you can in the thyroidectomized animal?

Taurog. The intact rat is quite a bit less sensitive than the thyroidectomized rat, even to thyroid hormone.

It is of interest, however, that in the absence of thyroid hormone one is able to restore the metabolic rate almost to normal, and if I had to venture a guess, I would say that the hydrocortisone is not acting at the same site that the thyroid hormone would be acting if it were injected. The rise in oxygen consumption of thyroidectomized rats receiving hydrocortisone may be mediated by biochemical reactions which differ in important respects from those reactions which are stimulated by the administration of thyroid hormone.

Pochin: What happens if you were to administer thyroxine to that thyroidectomized rat on cortisone when its metabolic rate has risen? Does it rise further or not?

Taurog: I hope I made it clear that this is not my study I was interested in this on hearing the people at the Institute of Experimental Biology speak of it, and I am afraid I am not in a position to give you further details.

At the Institute they have also been studying the effect of the ablation of various endocrine organs on metabolic rate. Another figure shows the results which they obtained with groups of rats that were either hypophysectomized, thyroidectomized, adrenalectomized or ovariectomized. Again, metabolic rates, in [illegible] r sq. metre per hour, are plotted [illegible] I thought it was of [illegible]

[illegible] of equal

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Taurog: I do not know if their nutrition was controlled

Barker: Do you know if the adrenalectomized animals were maintained on high salt?

Taurog: I am sure that the adrenalectomized animals were [illegible]

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his staff, and the Ciba Foundation for their great hospitality and for the kindness they have shown us in the past few days. I think probably this remark has been made by all their Chairmen in all their closing remarks because the kindness shown by Dr. Wolstenholme's staff is so very evident in a great variety of ways.

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